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SO-CALLED MIXED TUMORS OF THE MAMMARY GLAND OF DOG AND MAN

WITH SPECIAL REFERENCE TO THE GENERAL PROBLEM OF CARTILAGE AND BONE FORMATION

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A striking discrepancy in comparative pathology that has been recognized but that has remained unexplained for more than a century is the remarkably high incidence of cartilage and bone in tumors of the mammary glands of dogs as contrasted with man.[1] Precise statistics on histologically studied material are scarcely available. Nieberle [2] reported that 75 per cent of the neoplasms of breasts of dogs are mixed tumors in which cartilage is "frequently" present and bone somewhat less frequently. Of the 28 tumors described by Glendining,[1g] cartilage or bone was found in 5, or approximately 18 per cent. Of 13 mammary tumors examined in this laboratory, cartilage or bone was present in 4, or about 30 per cent. On the other hand, mammary tumors containing cartilage or bone are rare in man (Wilms [3]; Thinnes [4]; Sehrt [1h]). Gross [5] stated in his monograph that he had seen only 1 case of

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1. (*a*) Virchow, R.: Die krankhaften Geschwülste, Berlin, A. Hirschwald, 1863, vol. 1, pp. 481, 484, 507, 520 and 524. (*b*) Billroth, T.: Virchows Arch. f. path. Anat. **18**:51, 1860. (*c*) Labbé, L., and Coÿne, P.: Traité des tumeurs bénignes du sein, Paris, G. Masson, 1876, pp. 330-331. (*d*) Cornil, V., and Petit, G.: Bull. Soc. anat. de Paris **80**:23, 1905. (*e*) Kitt, T.: Text-Book of Comparative General Pathology, translated by W. W. Cadbury, Chicago, Chicago Medical Book Company, 1906. (*f*) Frei, W. E., in Joest, E.: Handbuch der speziellen pathologischen Anatomie der Haustiere, Berlin, R. Schoetz, 1925, vol. 4, p. 74. (*g*) Glendining, B.: Arch. Middlesex Hosp. **19**:198, 1910. (*h*) Sehrt, E.: Beitr. z. klin. Chir. **55**:574, 1907. (*i*) Ewing, J.: Neoplastic Diseases, ed. 2, Philadelphia, W. B. Saunders Company, 1928, pp. 538, 773-775 and 1018-1019.

2. Nieberle, K.: Ztschr. f. Krebsforsch. **39**:113, 1933.

3. Wilms, M.: Die Mischgeschwülste: III. Mischgeschwülste der Brustdrüse, Leipzig, A. Georgi, 1902.

4. Thinnes, H.: Virchows Arch. f. path. Anat. **264**:150, 1927.

5. Gross, S. W.: A Practical Treatise on Tumors of the Mammary Gland, New York, D. Appleton and Company, 1880, p. 53.

ossification of the breast, that of an ossifying fibroma. Labbé and Coÿne [1c] stated that they had never observed such tumors and insisted that those described in the literature (up to 1876) were all disputable. Cheattle and Cutler [6] mentioned that in their entire experience they had seen only 2 examples of cartilage in the human breast. Heuter and Karrenstein [7] in 1906 collected 5 instances of chondrosarcoma and 3 of osteosarcoma from the literature. In 1927 Thinnes [4] recognized the cases of only eight authors as definitely instances of osteochondrosarcoma. Gömöri [8] in 1936 was able to collect from the literature only 6 instances of mammary tumor with "well-developed bone tissue as a characteristic element." Although the actual number of such tumors is undoubtedly greater than the citations indicate, nevertheless, their relative rarity remains unquestioned.

Mammary tumors containing cartilage and bone are uncommon also in the horse, cow, goat and sheep (Frei [1f]). In reports of observations made on hundreds of spontaneous mammary tumors of mice by Haddow [9] and Haaland,[10] no mention is made of the presence of cartilage or bone. Murray [11] recorded a single cartilaginous tumor among 87 such growths. However, in a series of 87 spontaneous sarcomas of various organs of mice, Slye, Holmes and Wells [12] found 4 "subcutaneous or mammary" tumors containing cartilage. In experiments with a transplantable carcinoma of mice, which is described as having undergone sarcomatous transformation, Woglom [13] observed the presence of cartilage in 3 of the 4 mammary tumors in the second generation. In regard to rats, Bullock and Curtis [14] found no cartilage or bone in 94 spontaneous tumors. It may be safely concluded, therefore, that the incidence of cartilage and bone in tumors of mammary glands is peculiarly high in dogs.

There are, to be sure, other discrepancies in comparative oncology which lack adequate explanations. It is well known, for example, that tumors of the lip, esophagus, stomach (except the series of tumors of rats reported by Bullock and Curtis) and rectum are relatively rare in animals.[15] This is in strong contrast to their frequency in man.

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6. Cheattle, L., and Cutler, M.: Tumors of the Breast, Philadelphia, J. B. Lippincott Company, 1932, p. 485.
7. Heuter, C., and Karrenstein: Virchows Arch. f. path. Anat. **183**:495, 1906.
8. Gömöri, G.: Am. J. Surg. **33**:150, 1936.
9. Haddow, A.: J. Path. & Bact. **47**:553, 1938.
10. Haaland, M.: Scient. Rep. Invest. Imp. Cancer Research Fund **4**:1, 1911.
11. Murray, J. A.: Scient. Rep. Invest. Imp. Cancer Research Fund **3**:41, 1908.
12. Slye, M.; Holmes, H. F., and Wells, H. G.: J. Cancer Research **2**:1, 1917.
13. Woglom, W. H.: J. Cancer Research **3**:47, 1918.
14. Bullock, F. D., and Curtis, M. R.: J. Cancer Research **14**:11, 1930.
15. Dobberstein, J.: Virchows Arch. f. path. Anat. **302**:1, 1938. Wells, H. G.; Slye, M., and Holmes, H. F.: Am. J. Cancer **33**:223, 1938. Moschkowitz, L., and Sprinz, H.: ibid. **38**:271, 1940. Kitt.[1e] Slye, Holmes and Wells.[12]

It is felt that in the explanation of such differences there must lie facts of fundamental oncologic and possible histologic importance, particularly as relates to the much controverted subjects of chondrogenesis and osteogenesis. It was therefore decided to reconsider the subject when the opportunity for studying these mammary tumors presented itself. Those who have written on the presence of cartilage and bone in the breast have generally been concerned not with the reasons for the high incidence in dogs but with the genesis of these tissues. The purpose of this communication is therefore twofold:

1. To attempt to trace the genesis of cartilage and bone in these tumors.

2. To endeavor to isolate those factors peculiar to the dog which may be responsible for the unique incidence of cartilage and bone–containing mammary tumors in that species.

MATERIAL AND METHODS

The material to be considered includes 4 mammary tumors from dogs and 1 such tumor from a woman. In addition, because of certain features of the genesis of the cartilage and bone, 5 cases of gelatinous carcinoma of the human female breast are briefly analyzed.

The following stains were used: hematoxylin and eosin, Weigert and Van Gieson, Mallory's aniline blue, Best's carmine, Mayer's mucicarmine, Bielschowsky's silver, toluidine blue, iron and sudan. The tissues were subjected to pancreatic and tryptic digestion for aid in the differentiation of collagen and reticulum from mucus and fibrin.

TUMOR FROM DOG 322

Macroscopic Observations.—The specimen was removed surgically from a 7½ year old female Russian wolfhound. The tumor consists of a roughly spherical, moderately firm mass about the size of a grapefruit, measuring 14 cm. in diameter. The mass is covered by easily cleaved, apparently uninvolved skin except along the resected surface, which is formed by tumor. The sectioned surfaces present a variegated appearance. They are for the most part grayish tan mottled irregularly with pale to slate gray tissue, in which are scattered small areas of deep brown pigment. Numerous cysts measuring up to 3 cm. in diameter are present. On close examination, one is able to distinguish several small gray irregular foci, which have the consistency of cartilage.

Microscopic Observations.—The sections present a very heterogeneous picture. The tumor is composed, for the most part, of irregular nodules of glands surrounded by varying widths of fibrous tissue, which occasionally appear compressed into pseudocapsules. In places, this stroma is invaded, with consequent disappearance of part of the capsule and coalescence of adjacent nodules. Scattered in the stroma are several small abscesses, as well as numerous macrophages filled with fat, carminophilic droplets and iron pigment.

The glands are highly irregular in size and shape. Some are filled with a homogeneous, apparently albuminous eosinophilic secretion, which does not take the carmine or toluidine blue stain and in which macrophages and polymorphonuclear leukocytes are frequent. Many of the glands are lined by stratified layers of

columnar cells with large, moderately vesicular nuclei showing little atypism. One is frequently able to make out an incomplete layer of "basket" or myoepithelial cells along the outer border of the glandular epithelium, just adjacent to the basement membrane. The epithelium of many of the glands forms stalklike invaginations with delicate central fibrous cores. Some of the glands are partially to completely filled with compact cellular masses.

Of particular interest are those glands whose innermost layer of epithelium is just beginning to loosen and to separate from the adjacent cells. In these, one frequently sees carminophilic droplets, both in the cytoplasm and in the intercellular fluid. The intercellular spaces become progressively wider, and the

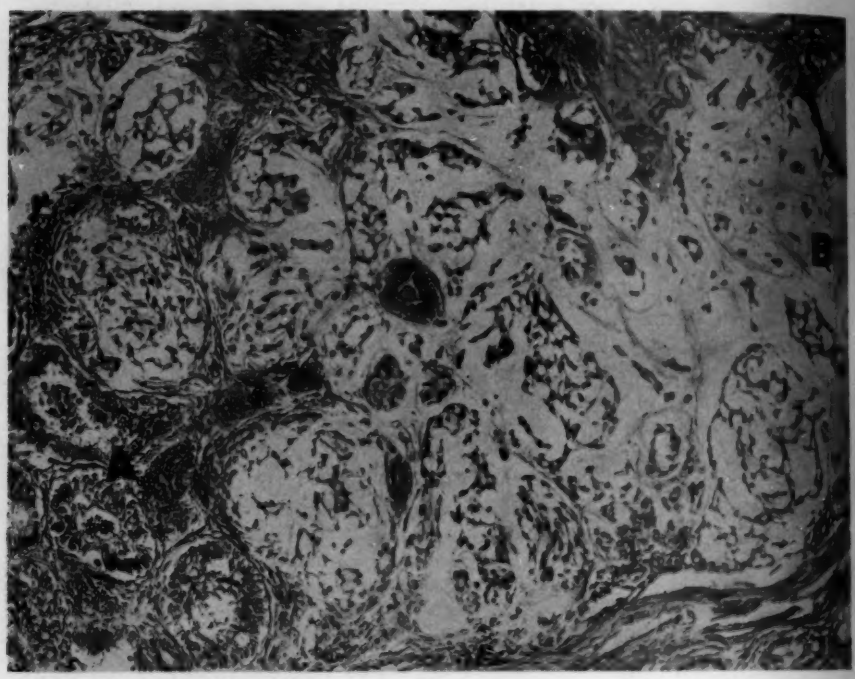

Fig. 1 (dog 322).—This section illustrates several of the early transitional stages in the development of cartilage from epithelium. Note the gradual separation of the *adult* epithelium first into a retiform pattern and finally into completely isolated single cells, pairs or small clumps. As with normal epiphysial cartilage, both the matrix and the cells exhibit an increase in carminophilia, as well as metachromasia with toluidine blue, with the progression of the transition from the compact epithelium at *A* to the isolated cells at *B*. Hematoxylin and eosin.

cells appear pushed farther and farther apart, with resultant gradual encroachment on, and finally obliteration of, the lumen of the gland. In short, what was once stratified epithelium cuffing a lumen has become a delicate laciform tracery (fig. 1). Where the intercellular fluid has accumulated in sufficient quantity, this reticular network originating from the glandular epithelial lining is forced apart, and the cells become isolated in a mucoid matrix (figs. 2, 3 and 4). In the

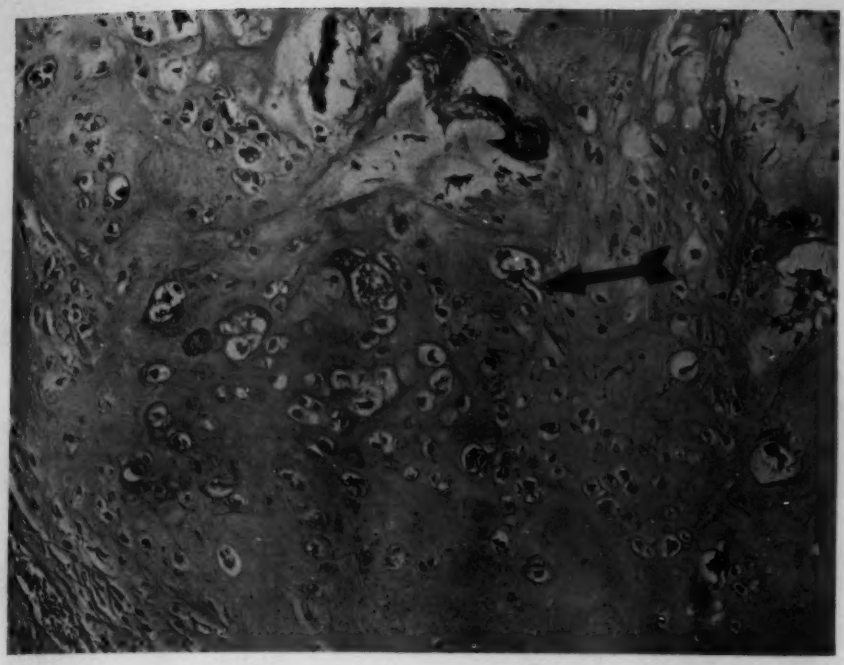

Fig. 2 (dog 322).—This field demonstrates clearly the mechanism of isolation of the originally adult acinar epithelium within the mucocollagenous matrix and the end stages of the transformation into cartilage. The arrow points to a cell about to be enclosed by matrix. Hematoxylin and eosin.

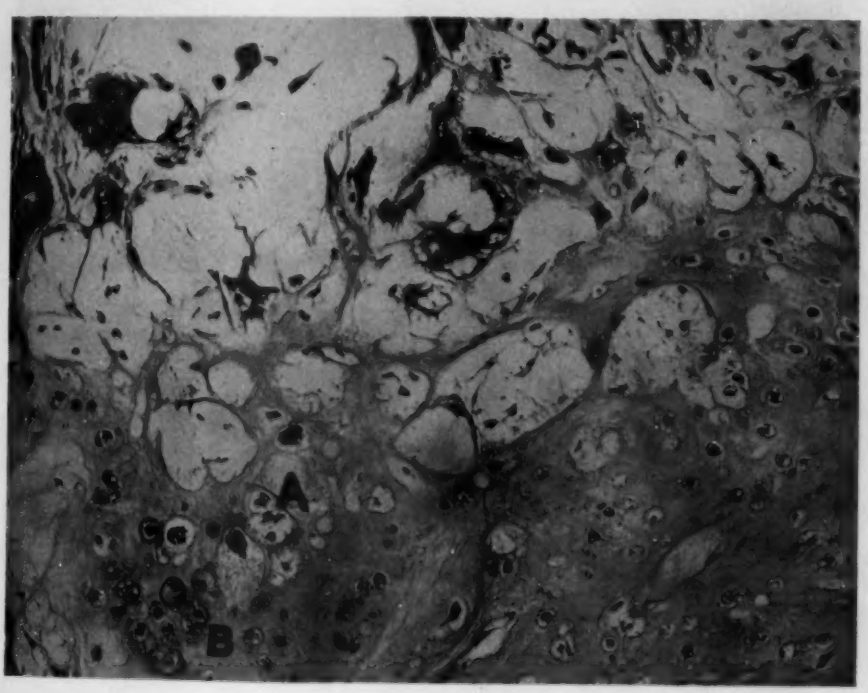

Fig. 3 (dog 322).—This section illustrates the transition of epithelium into cartilage by the process of disruption of the acinar epithelium (*A*) within the mucocollagenous matrix. The cells in the end stage (*B*) are indistinguishable from chondrocytes. Hematoxylin and eosin.

meantime the cells have become spindle shaped and often are filled with carminophilic droplets. The matrix in which they are embedded generally stains only faint pink with carmine or mucicarmine but manifest definite metachromasia with toluidine blue. The basket cells appear to suffer the same fate as the remainder of the epithelial lining, and they, too, come to lie loose in the fluid matrix. However, one does not observe selective proliferation of these cells. The normal ratio between these myoepithelial cells and the remaining epithelial cells is at most maintained and is frequently diminished.

Scattered through the matrix are partially fragmented fibers which stain pink to red with acid fuchsin, pale blue to deep blue with aniline blue and yellowish brown to black with silver and which for the most part resist tryptic and pancreatic

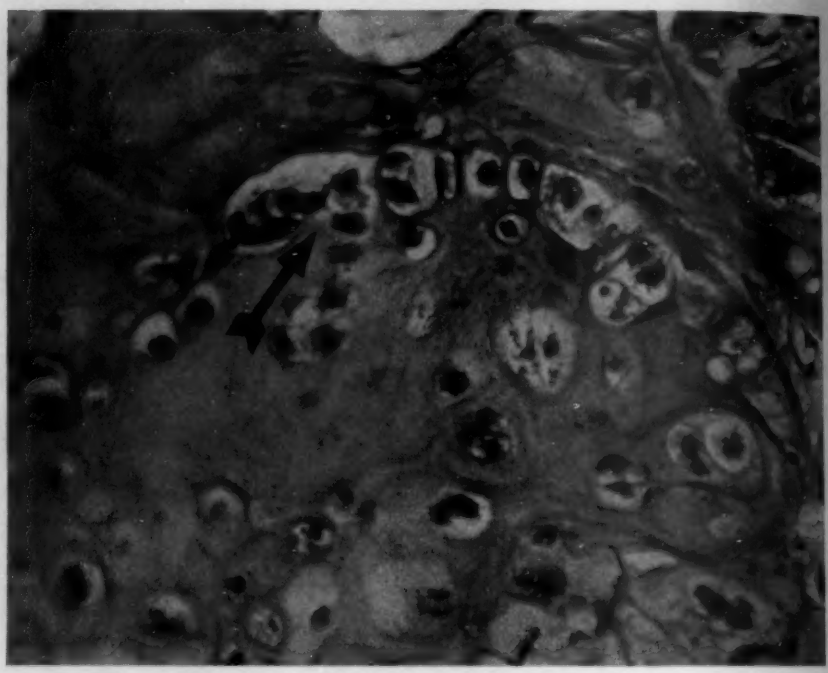

Fig. 4 (dog 322).—High power magnification of a cartilaginous focus, illustrating the mechanism of isolation of epithelial cells by the matrix. The arrow points to a disrupting clump of acinar cells. Bielschowsky's silver stain.

digestion. They are therefore identifiable as collagenous and reticulum fibers. The source of the reticulum fibers appears most likely to be the argyrophilic basement membrane of the disrupted acini. Part of the collagenous tissue obviously arises from the surrounding stroma, since definite continuity is observed in many places. Collagenous fibers are seen also directly within the mucus, however, as if there has occurred collagenous transformation of the mucus, or, at any rate, partial replacement of the latter by collagenous fibers.

In those areas where the metachromasia is most pronounced, there are isolated epithelial cells partially surrounded by coves of matrix or actually completely enclosed in lacunae (figs. 2, 3 and 4). The cytoplasm of these cells contains

abundant carminophilic granules, as does the cytoplasm of chondrocytes. The cells are anchored to the lacunar walls by cytoplasmic strands, as if the remainder of the cell had shrunken. In some of the lacunae one sees, just inside of their capsules, granular eosinophilic material, which is found commonly in normal cartilage. Small droplets of fat are seen in some of the cells. In general, the nuclei appear viable, although here and there are imprisoned degenerating epithelial cells. Such foci are histologically indistinguishable from true hyaline cartilage. It is noted that the more closely these various portions of the tumor approach the histologic pattern of cartilage, the more carminophilic and metachromatic does

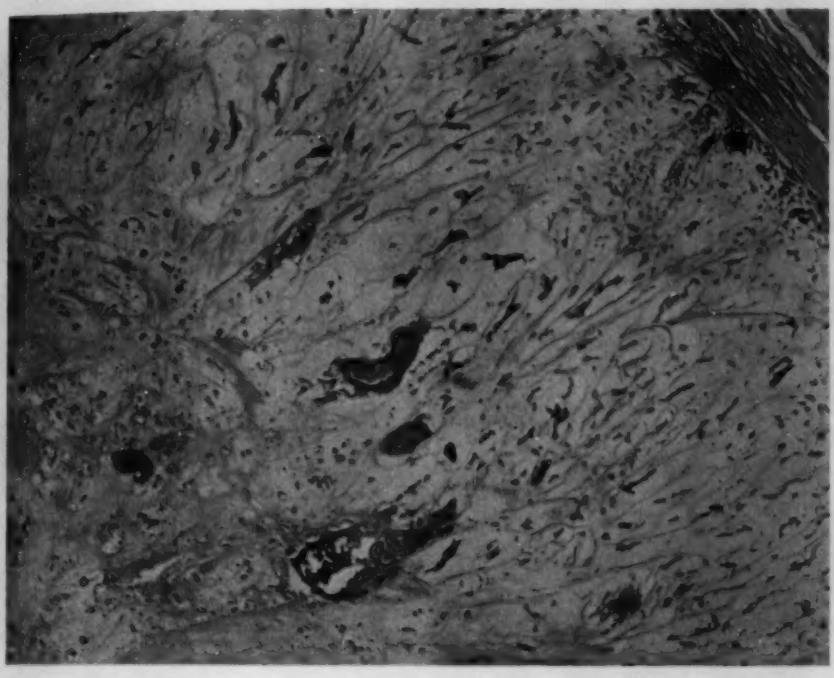

Fig. 5 (dog 322).—Note transition from right to left of epithelium to early cartilage by the process of dispersion and isolation of the epithelial cells in an increasingly carminophilic and metachromatic matrix. Hematoxylin and eosin.

the matrix become. In parallel manner, the enclosed cells stain progressively more deeply with carmine as the tissue takes on the appearance of cartilage.

Diagnosis.—The tumor is a myxochondroadenocarcinoma.

Comment.—This tumor is interpreted as representing an adenocarcinoma with foci of transformation of epithelial cells into myxomatous tissue and cartilage. In this process there has occurred a replacement of a mucoid fluid by collagenous tissue. It is impossible to be certain whether or not this replacement signifies direct conversion of mucus into collagen—a phenomenon acknowledged by Ehrich,[16]

16. Ehrich, E.: Beitr. z. klin. Chir. **51**:368, 1906.

von Ebner,[17] Ricker and Schwalb [18] and Ewing [11]—or derivation from preexisting collagen. It is significant that the progressively increasing carminophilia of the transition stages of the "epithelial cartilage" parallels that observed in the developing epiphysial cartilage (Harris [19]).

TUMOR FROM DOG 701

The specimen was removed from the mammary region of a stray adult female bitch of unknown age.

Macroscopic Observations.—The specimen consists of a spherical, moderately firm tumor mass about the size of a walnut (3 cm. in diameter), to which the

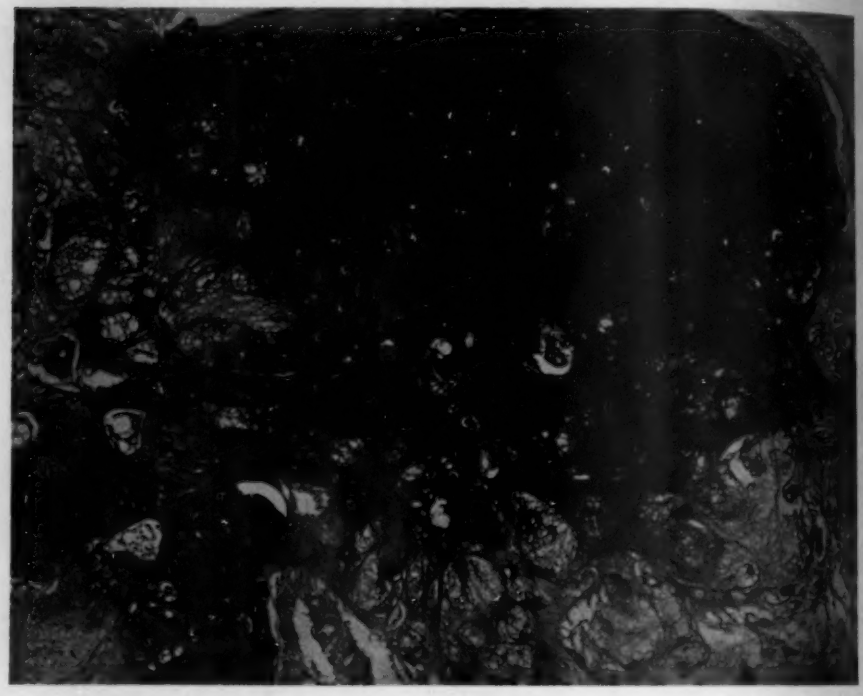

Fig. 6 (dog 322).—Nonencapsulated foci of cartilage. Note the increasing metachromasia as the epithelial tissue and its matrix progressively acquire the characteristics of cartilage. Toluidine blue.

overlying skin is adherent. The sectioned surface is pale purple gray mottled with bright yellow, 2 to 3 mm. flecks. Several areas varying from 4 to 8 mm. in diameter are light gray and have the consistency of cartilage. Two large cysts, measuring 1.2 by 0.5 and 1.5 by 0.8 cm., are present at the periphery.

Microscopic Observations.—Sections show the tissue to be composed of glands with marked variation in size and shape. Several areas are necrotic, hemorrhagic

17. von Ebner: Ztschr. f. Zool. **62**:469, 1896.

18. Ricker, G., and Schwalb, J.: Die Geschwülste der Hautdrüsen, Berlin, S. Karger, 1914, pp. 210-224.

19. Harris, H. A.: Nature, London **130**:996, 1932.

and loosely infiltrated with polymorphonuclear leukocytes. Irregular deposits of calcium are present in some of these necrotic areas. The glands are lined by columnar or polygonal cells containing large hyperchromatic nuclei with one or more prominent nucleoli. Mitotic figures are abundant. In many glands the epithelium is stratified or papillary and frequently fills the lumens. In several areas the epithelium is reticulated so as to simulate parts of the tumor of dog 322. In addition to the reticulated epithelium, the cells of which are separated by a homogeneous fluid, there are found glands in various stages of disruption lying loose in the eosinophilic, partially metachromatic (toluidine blue) matrix. As in the previous case, there are a few areas where one can easily follow the isolation of these cells within the mucoid matrix. There are, in addition, however, isolated spindle cells with vesicular nuclei which strongly resemble fibroblasts. The cytoplasm of both these types of cells, as they lie isolated in the matrix, is vacuolated and markedly carminophilic. Some of the cells lie in lacunae surrounded by "capsules," to which shreds of cytoplasm are adherent, as if the irregular spaces within the lacunae are the consequence of the cytoplasmic vacuolation. About some of the lacunae harboring intact cells, as well as near several clumps of cells not in lacunae but within matrix, there is a sprinkling of fine calcareous granules. An occasional nucleus in these regions is intensely basophilic, as if calcified. In those areas where chondrification has progressed, one observes an increasing affinity of the matrix for aniline blue and carmine, as well as distinct bluish (Mallory's aniline blue) bundles of collagenous fibers.

Diagnosis.—The tumor is a myxochondroadenocarcinoma.

Comment.—The cells taking part in the chondrification appear to be both epithelial and mesenchymal. Collagenous fibers within the matrix are obvious. It is interesting to note the affinity of the matrix for calcium as shown by the presence of the calcareous granules, particularly about lacunae containing intact cells. The affinity of the perilacunar matrix for calcium is exhibited generally in the calcification of ordinary osteoid tissue (Dibbelt [20]).

TUMOR FROM DOG 112

The animal was an 11 year old female Kerry blue terrier.

Macroscopic Observations.—The specimen consists of an oval encapsulated firm tumor mass, measuring 6 by 4 cm., removed surgically from the hindmost breast. The overlying skin is not adherent. The sectioned surface is pale purple gray mottled with yellow flecks and several small hemorrhagic areas. The consistency of the sectioned surface is similar to that of raw potato.

Microscopic Observations.—Sections reveal the tumor to be composed principally of hyaline cartilage and osteoid tissue merging with compact nests of polygonal cells with large hyperchromatic nuclei and prominent nucleoli. Mitotic figures are numerous. The cytoplasm of these cells is relatively abundant and occasionally tapers at either pole, particularly near the periphery of the cell nests. In these regions the cells appear to merge with the surrounding scanty stroma. In other portions the cells become separated by varying amounts of a hyaline eosinophilic material. The cells in these areas lie in lacunae as the matrix apparently inspissates, acquiring a bluish tint (hematoxylin and eosin) as well as a pronounced affinity for aniline blue. The cytoplasm of these cells is vacuolated, but the nuclei appear intact. The picture in these areas is indistinguishable from that of hyaline cartilage and includes the property of marked metachromasia. The carmine stain, however,

20. Dibbelt, W.: Beitr. z. path. Anat. u. z. allg. Path. **50**:411, 1911.

is negative, probably owing to delayed fixation. About the areas of osteoid and osseous tissue the tumor cells are frequently alined in a regular row so as to resemble osteoblasts. Numerous giant cells corresponding to osteoclasts are also present both at the periphery of these foci and within the cellular portions of the tumor. There are several areas of hemorrhage and necrosis with infiltration by polymorphonuclear leukocytes, lymphocytes and macrophages.

No actual gland formation is present within the tumor proper. However, at the periphery there are encapsulated nests of glands with marked papillary projections of epithelium which are not carcinomatous. Several cysts are present which are filled with an eosinophilic, nonmetachromatic secretion.

Diagnosis.—The tumor is an osteochondrosarcoma.

Comment.—The cells within the chondral, osteoid and osseous tissue appear to be mesenchymal tumor cells.

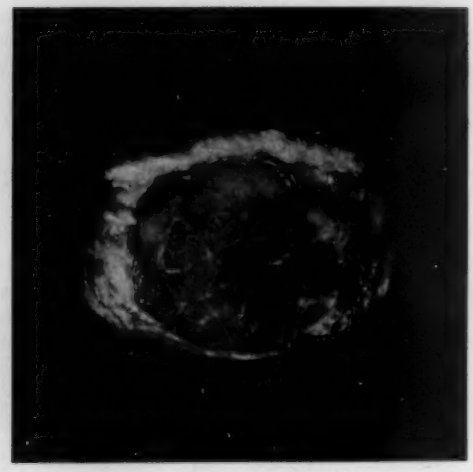

Fig. 7 (dog 166).—Chondroadenocarcinoma of a mammary gland with stromal chondrification and ossification. Note the encapsulation and the location directly beneath the skin. Trauma to this mammary gland is obviously borne almost wholly by the tumor.

TUMOR FROM DOG 166

This dog was an adult bitch; the exact age is unknown.

Macroscopic Observations.—The specimen consists of a tumor removed at autopsy from the left caudal mammary gland. It is a firm walnut-sized mass (3 by 2.5 cm.), completely enclosed in a thin fibrous capsule. The cut surface presents a motley of colors, including the deep purple-brown of old hemorrhage interspersed with irregular areas of grayish white and yellowish tan. The latter areas grate as if composed of bone.

Microscopic Observations.—Sections reveal the tumor to be composed predominantly of nests of irregularly patterned glands of varying sizes and shapes. These are lined for the most part by a single layer of columnar cells with vesicular nuclei containing prominent nucleoli. Mitotic figures are absent. Occasionally the glands are partially occluded by projections or stratifications of epithelium. Here and there the lumen of a gland is completely obliterated by epithelium, which

has become reticulated as if fluid had accumulated between the individual cells and forced them apart. These epithelial cells appear progressively to acquire a more definite spindle shape so as to simulate within their fluid matrix a myxomatous tissue. In several areas, each about the size of a low power field, the cells no longer are spindle shaped but appear to have drawn in their cytoplasmic processes and to have become isolated in a matrix. Their cytoplasm is vacuolated and slightly carminophilic. Capsular rims are present about them, thereby simulating chondral tissue. The matrix takes the Van Gieson and Mallory stains for collagen and is not digested by pancreatin. As with ordinary cartilage, the metachromasia of these portions of the tumor is intense. In other parts, irregular trabeculae of

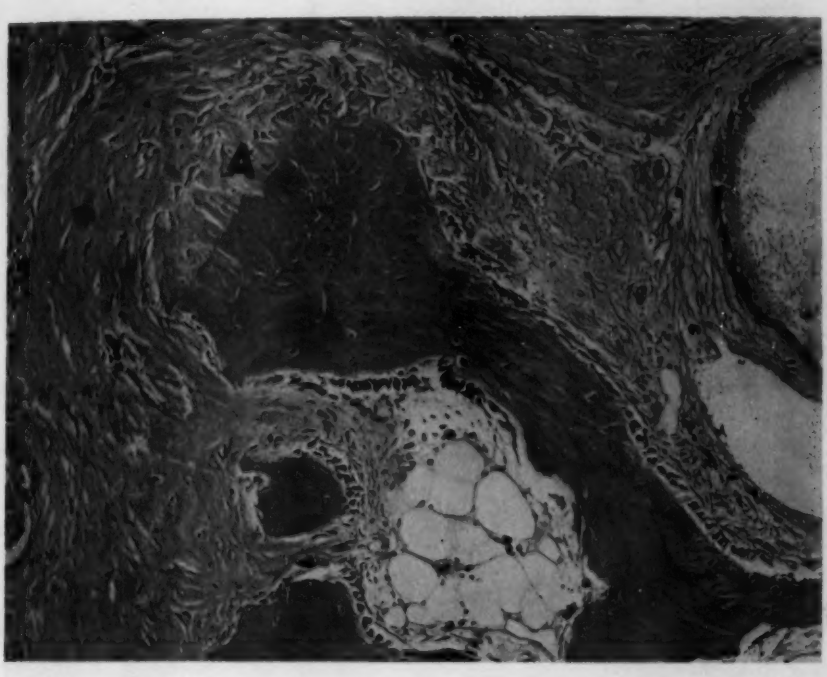

Fig. 8 (dog 166).—Bony trabecula in stroma. Note at *A* the apparent advancing line of ossificaiton and the absence of osteoblasts in this region where, according to the current concept of the secretion or transformation of the ectoplasm of osteoblasts, they ought logically to be found. Instead, this seems to represent a direct transformation of *mature* fibrous tissue with incidental inclusion of cells (osteocytes). Hematoxylin and eosin.

mature bone are found. These show typical laminations, rims of osteoblasts, scattered osteoclasts and a fatty "marrow" with small islets of cells, which are predominantly polymorphonuclear leukocytes. Some of the bony trabeculae merge with small foci of hyaline cartilage. This transition, which appears to be graduai in sections stained with hematoxylin and eosin, is seen to be sharp in those stained with toluidine blue. In these the intensely metachromatic cartilage is contrasted with the pale blue-staining adjacent bone. The osteoid and osseous tissue appears in places to be directly continuous with the collagen of the stroma as if there

had taken place a direct transformation therefrom. Furthermore, about parts of some of the trabeculae, osteoblasts are absent (figs. 8 and 9). There is also much evidence of old hemorrhage in the stroma in the form of numerous collections of macrophages filled with iron pigment, as well as scattered foci showing acute and chronic inflammatory reaction.

Diagnosis.—The tumor is a chondroadenocarcinoma with stromal chondrification and ossification.

Comment.—This tumor is interpreted as an adenocarcinoma with cartilage derived from epithelium as well as from stroma. Bone, formed apparently by the direct ossification of the stroma, is also present.

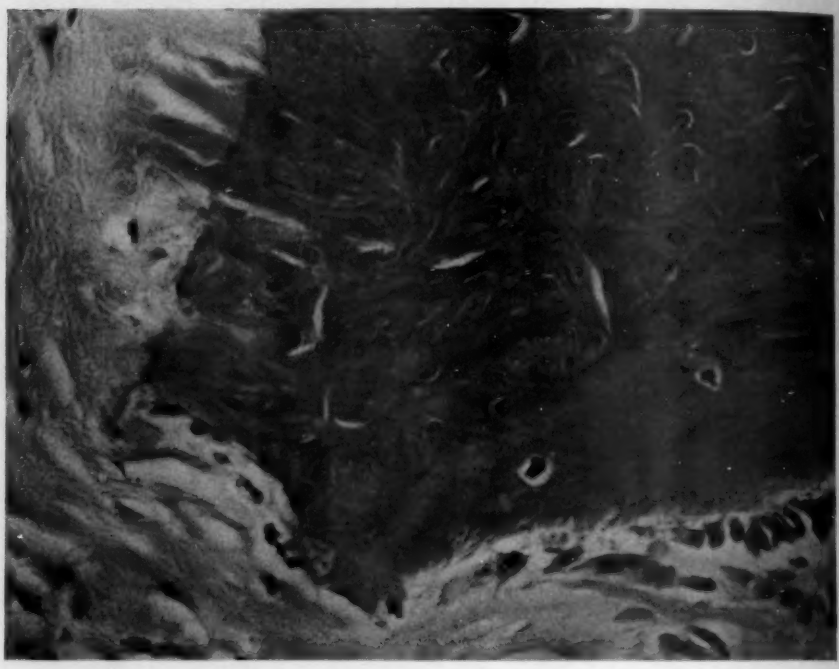

Fig. 9.—Higher magnification of part of figure 8. Note the continuity at the advancing line of ossification of the collagenous fibers into the bony matrix. Again, note the absence of a rim of osteoblasts at this site of progressing ossification. Hematoxylin and eosin.

TUMOR OF HUMAN FEMALE BREAST

A white woman, aged 28, married, was admitted to the Mount Sinai Hospital, Oct. 26, 1921. She had 2 children, the last born one year before. A physician had noted a mass about the size of an egg, two and a half years before admission. The tumor grew progressively, with particularly rapid enlargement during the month prior to admission.

The patient was well nourished and developed. Examination of the left breast revealed diffuse enlargement to several times the size of the opposite breast. The overlying skin was smooth, glossy and discolored purple-red. Several

whitish drops of fluid could be expressed through the everted nipple of the involved breast. The tumor was not adherent to the underlying fascia.

Macroscopic Observations.—The specimen consists of a left breast removed by radical resection. The overlying skin is discolored purple-red but is not retracted. The nipple is everted. Replacing most of the parenchyma is an encapsulated globular cystic tumor, measuring 20 cm. in diameter. Its sectioned surface is purple-gray and presents numerous cystic areas filled with gelatinous fluid. The lining of these cysts is papillomatous. The intervening tissue is moderately firm, smooth and purple-gray with scattered whitish areas as well as areas of frank necrosis.

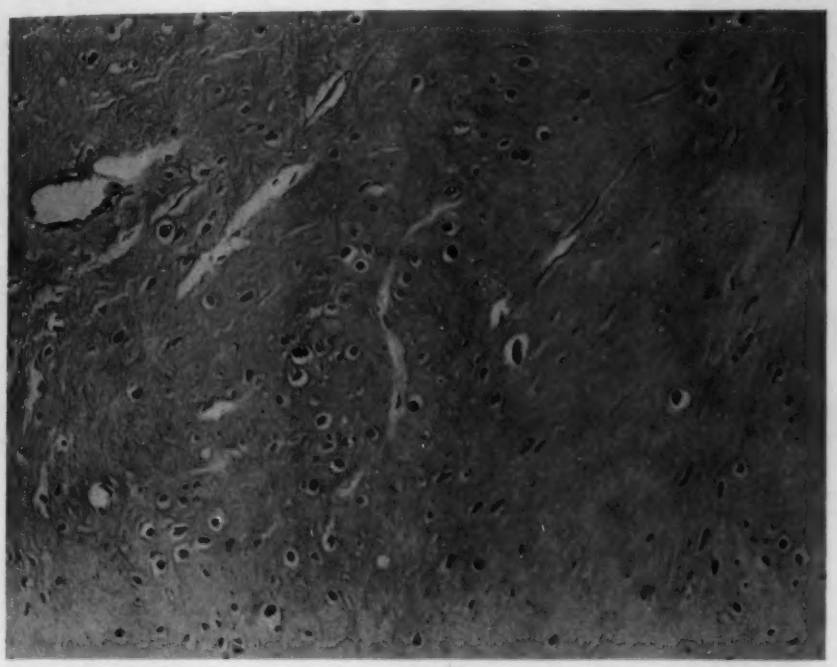

Fig. 10.—Fibromyxochondrosarcoma of the human breast. This field shows an area of fibrocartilage resulting from metaplasia of *adult, sclerotic* collagenous tissue. Note early formation of lacunae and later transitional stages. Hematoxylin and eosin.

Microscopic Observations.—Sections reveal the tissue to be composed almost exclusively of collagenous tissue, in which a few single mammary acini are isolated. The fibrous tissue is for the most part markedly cellular. The nuclei show moderate hyperchromatism and irregularity of size and shape. Mitotic figures are not seen. These compact spindle cells frequently merge, on the one hand, with a loose fibrillar, in places myxomatous tissue and, on the other, with a more homogeneous sclerotic tissue, in which the cells are isolated in lacunae so as to present the picture of fibrocartilage. On closer examination, one readily observes the transition of the cellular fibrous tissue to a more sclerotic type and thence

to fibrocartilage without the intervention of the myxomatous tissue. There is *no transition between the myxomatous tissue and the fibrocartilage.* In this process of chondrification the spindle-shaped nuclei with their rim of cytoplasm (fibrocytes) are seen to be isolated from the collagen and come to lie in an oval alcove surrounded by collagenous fibrils. The cell then appears to curl on itself. The alcove becomes circular and forms a typical lacuna, the rim of which stains more intensely than the surrounding fibers, so as to resemble a capsule. At the same time the rim of cytoplasm becomes vacuolated and shrinks, possibly owing to fixation. Some of the lacunae are occupied by two cells, a few of which appear fused, while others are evenly divided by a septum. The glycogen stain is negative. (The specimen is eighteen years old.)

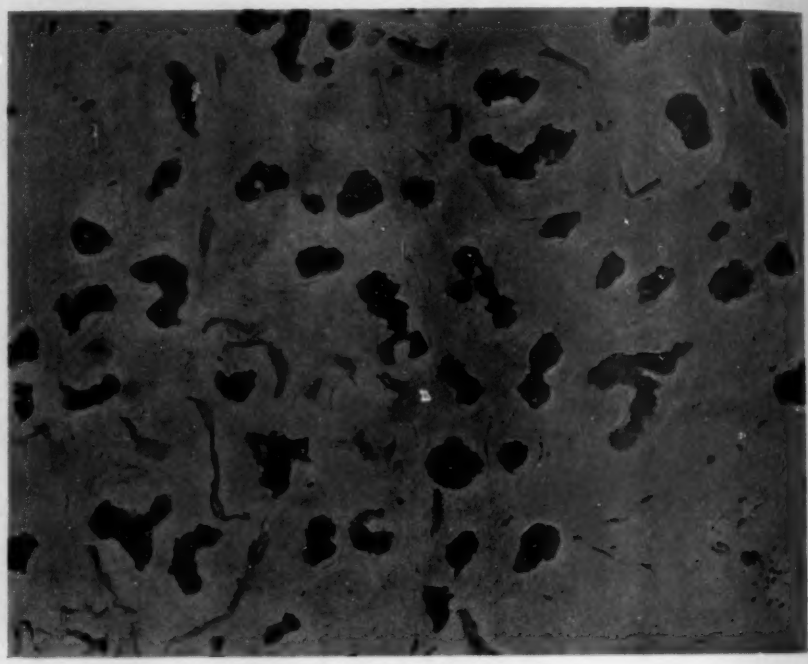

Fig. 11.—Colloid carcinoma of the human breast. Contrast the nests of cells in this tumor with the retiform pattern found in the canine tumors. Hematoxylin and eosin.

Several isolated glands show no evidence of proliferation. They are found in all portions of the tumor, including the fibrocartilage.

The lymph nodes are uninvolved.

Diagnosis.—The tumor is a fibromyxochondrosarcoma.

Comment.—This tumor represents a sarcoma with transformation of the sclerotic, collagenous portion into fibrocartilage. It is generally stated that fibrocartilage develops directly from immature mesenchymal cells endowed with an inherent tendency in that direction. Such a vitalistic concept precludes the forma-

tion of fibrocartilage *directly* from mature collagenous tissue such as one observes in this tumor. It is true that this sclerotic tissue is part of a tumor, and it might conceivably be argued that the cells may have acquired fibroblastic potencies by dedifferentiation. However, it is the sclerotic, mature, fibromatous portion of the tumor which manifests an easily traced transition to fibrocartilage, rather than the cellular or even myxomatous tissue.

In summary, cartilage was found in 4 canine mammary tumors. In addition, osteoid tissue was present in one of these and bone in another. It appears that the cartilage in 3 of the tumors is epithelial in orgin, notwithstanding the orthodox concept of the specificity of germ layers. It is emphasized that this epithelium is ectodermal in origin and that it is not embryonic but typical of the adult type. This epithelial metamorphosis seems to be a mechanistic[21] process, depending on physicochemical phenomena rather than on vitalistic properties with which the cells are inherently endowed. Part of the cartilage in 2 of the tumors, and the osteoid and osseous tissue as well, are considered mesenchymal in origin. The fibrocartilage of the tumor of the human breast appears to arise directly from the mature collagenous tissue rather than from immature mesenchymal cells with cartilaginous propensities. This metamorphosis, too, is regarded as physicochemical in nature and without relationship to specific cellular endowments.

THEORIES AS TO THE GENESIS OF CARTILAGE AND BONE IN SO-CALLED MIXED TUMORS OF THE BREAST

The literature on the subject of the genesis of cartilage and bone in so-called mixed tumors of the breast and other organs is highly complicated and controversial. The principal theories that have been advanced—both for human and canine tumors—are based on the following concepts:

1. Embryonic rests.[22]

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21. The term "mechanistic" as used in this report is contrasted with "vitalistic" and is applied in the sense of a physicochemical explanation of biologic phenomena. See discussion by M. R. Cohen in his "Reason and Nature" (New York, Harcourt Brace & Company, Inc., 1931, pp. 240-292).

22. (*a*) Cohnheim, J.: Vorlesungen über allgemeine Pathologie, Berlin, A. Hirschwald, 1877, vol. 2, chap. 7. (*b*) Leser: Beitr. z. path. Anat. u. z. allg. Path. **2**:379, 1888. (*c*) Lecène, in discussion on Peyron, M. A.: Bull. Assoc. franç. p. l'étude du cancer **13**:349, 1924. (*d*) Ribbert, H.: Geschwulstlehre, Bonn, F. Cohen, 1904. (*e*) Borst, M.: Echte Geschwülste, in Aschoff, L.: Pathologische Anatomie, ed. 8, Jena, Gustav Fischer, 1936, vol. 1 chap. 9. (*f*) St. Arnold: Virchows Arch. f. path. Anat. **148**:449, 1897. (*g*) MacCallum, W. G.: A Text-Book of Pathology, ed. 6, Philadelphia, W. B. Saunders Company, 1936, pp. 1193-1194. (*h*) Wilms.[2] (*i*) Heuter and Karrenstein.[7] (*j*) Gömöri.[8]

2. Local or autochthonous development.

(*a*) Connective tissue metaplasia.[23]

(*b*) Epithelial metaplasia.[24]

The authors supporting the theory based on this concept account for at least a portion of the cartilage (or "pseudocartilage," as some call it [see page 607]) by epithelial transformation.

1. *Embryonic Rests.*—The hypothesis that an embryonic rest of cartilage or bone is stimulated to neoplasia after a period of latency is convenient, simple to grasp and, in blanket fashion, skirts the possibilities of intricate cellular transformations. In its favor are said to be (1) the wide diversity of tissues that may occur in a single mixed tumor of the breast and (2) their frequent encapsulation. The source of these skeletogenous rests has been attributed to the ribs or clavicle (Arnold [22f]). This general hypothesis accounting for heterotopic chondral or osseous tissues through dysontogenesis has met with much opposition when applied to a site theoretically more favorable for such occurrences—for example, the parotid region (Krompecher [25]; Masson and Peyron [24a]; Fraser [26]). At least here the more complicated development and the proximity of the cartilage of the branchial arches to the parotid region afford for the adherents to the hypothesis advanced by Cohnheim [22a] a possible basis for the occurrence of misplaced cartilaginous rests. In the breast this possibility based on unappropriated particles of the anlages of the rib or the clavicle would certainly seem to be very much less. Furthermore, these rests would be obliged to occur much more frequently in the canine than in the human breast in view of the greater incidence of such tumors in the former. If in the process of formation of ribs there is a developmental phenomenon peculiar to dogs which permits of frequent displacement of an anlage, it has not been demonstrated.

Moreover, one can hardly reasonably reconcile such a hypothesis with the finding of mammary acinar tissue directly in the midst of an area of cartilage

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23. (*a*) Stilling, H.: Deutsche Ztschr. f. Chir. **15**:247, 1881. (*b*) Bowlby, A. A.: Tr. Path. Soc. London **33**:306, 1883. (*c*) Edelman, H.: Beitr. z. path. Anat. u. z. allg. Path. **78**:618, 1927. (*d*) von Hacker: Arch. f. klin. Chir. **27**:614, 1882. (*e*) Chrier, L., and Deval, C.: Bull. Soc. anat. de Paris **85**:586, 1910. (*f*) Fry, H. J. B.: J. Path. & Bact. **36**:529, 1927. (*g*) Auler, H., and Wernicke: Ztschr. f. Krebsforsch. **36**:529, 1927. (*h*) Durante, in discussion on Peyron, M. A.: Bull. Assoc. franç. p. l'étude du cancer **13**:349, 1924. (*i*) Cornil, V.: Les tumeurs du sein, Paris, F. Alcan, 1908. (*j*) Davidsohn, C.: Ztschr. f. Gynäk. **35**:1357, 1909. (*k*) Busser, F.: Ann. d'anat. path. **6**:1247, 1929. (*l*) Freese, K.: Ztschr. f. Tiermed. **9**:206, 1905. (*m*) Imomaki, K.: Gann **26**:1, 1932. (*n*) Pitschugin, L. M.: Virchows Arch. f. path. Anat. **280**:136, 1931. (*o*) Coats, J.: Glasgow M. J. **4**:35, 1871. (*p*) Virchow.[1a] (*q*) Billroth.[1b] (*r*) Sehrt.[1h] (*s*) Kitt.[1e] (*t*) Thinnes.[4] (*u*) Frei.[1f]

24. (*a*) Masson, P., and Peyron, M. A.: Bull. Assoc. franç. p. l'étude du cancer **7**:219, 1914. (*b*) Masson, P.: ibid. **11**:345, 1922; (*c*) Atlas du cancer, Paris, Association française pour l'étude du cancer, 1924, pts. 3 and 4; (*d*) Diagnostics de laboratoire: II. Tumeurs, Paris, A. Maloine et fils, 1923, p. 309. (*e*) Tudhope, G. R.: J. Path. & Bact. **48**:499, 1939. (*f*) Nieberle.[2] (*g*) Ehmann, quoted by Nieberle.[2] (*h*) Ewing.[11]

25. Krompecher, E.: Beitr. z. path. Anat. u. z. allg. Path. **44**:51 and 87, 1908.

26. Fraser, A.: Surg., Gynec. & Obst. **27**:19, 1918.

or, in other words, in the midst of an embryonic rest (Petit[27]). This is clearly demonstrated in the material presented herewith. In addition, some of the cartilaginous foci are not encapsulated but merge with the surrounding tissue. The mere encapsulation of the cartilage or the presence of a heterogeneity of tissues need not be evidence of dysontogenesis, as will be explained shortly on the basis of epithelial transformation. For these reasons, in addition to those based on histologic studies, the Cohnheim theory, popularized by Wilms[8] in the early part of this century, appears to be giving ground to the hypotheses based on local or autochthonous development of cartilage and bone not only in tumors of the breast but also in those of the parotid region.

2. *Local or Autochthonous Development.*—(*a*) Connective Tissue Metaplasia or Development from Separate Germ Layers: One of the currently advocated hypotheses simplifies the problem somewhat by ascribing the origin of the epithelial and mesenchymal components to the neoplasia of two germ layers; that is to say, the carcinomatous portion is derived from epithelium, and the sarcomatous portion, including the cartilage and bone, is a transformation of mesenchymal tissue. A modification of this states that the proliferating epithelium stimulates the neighboring stroma to acquire embryonic potencies with the capacity, accordingly, to develop into a variety of mesenchymal derivatives, including cartilage and bone (Auler and Wernicke[23g]). This is compatible with the hypothesis of Ehrlich and Apolant[28] to the effect that the transformation of the transplantable carcinoma of mice into sarcoma is due to a stimulation of the stroma by the proliferating epithelium. Auler and Wernicke[23g] suggested that the necrotic epithelium may furnish the inciting factor.

There seems to be no doubt that cartilage or bone may replace connective tissue. The histologic mechanism, however, is controvertible and will be further considered on page 621. It is nevertheless a fact that such heterotopic bone formation has been found in almost every mammalian organ, including the blood vessels, heart, lungs, fallopian tubes and uterus, in hematomas, in abdominal wounds and in the stroma of tumors, for example, tumors of the ovary, cecum, rectum and other tissues.[29] There is no reason, therefore, to doubt the possibility of a replacement of stromal tissue by cartilage or bone in tumors of the breast. A second source of the cartilage and bone is, of course, considered to be the mesenchymal tumor cells themselves.

(*b*) Epithelial Metaplasia: Contrary to the concept of the specificity of germ layers, it is maintained by some observers that the epithelial cells of certain tumors may give rise to cartilage. According to Lang,[30] this thought was advanced

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27. Petit, M. G.: Bull. Soc. anat. de Paris **81**:373, 1906.

28. Ehrlich, R., and Apolant, H.: Centralbl. f. allg. Path. u. path. Anat. **17**: 513, 1906.

29. (*a*) Mönckeberg, J. G.: Virchows Arch. f. path. Anat. **167**:191, 1902. (*b*) Bunting, C. H.: J. Exper. Med. **8**:365, 1906. (*c*) Buerger, L., and Oppenheim, A.: ibid. **10**:354, 1908. (*d*) Pollack, K.: Virchows Arch. f. path. Anat. **165**:129, 1901. (*e*) Nicholson, G. W.: J. Path. & Bact. **21**:287, 1917. (*f*) Poscharissky, J. F.: Beitr. z. path. Anat. u. z. allg. Path. **38**:135, 1905. (*g*) Asami, G.: Am. J. M. Sc. **160**:107, 1920. (*h*) Leriche, R., and Policard, A.: Les problèmes de la physiologie normale et pathologique de l'os, Paris, Masson & Cie, 1926. (*i*) Chauvin, E., and Rouslacroix, A.: J. d'urol. **27**:465, 1929.

30. Lang, F. J., in Henke, F., and Lubarsch, O.: Handbuch der speziellen pathologischen Anatomie und Histologie, Berlin, Julius Springer, 1929, vol. 5, pt. 2, p. 169.

as early as 1859 by von Bruns and has since been applied almost exclusively to the so-called mixed tumors of the salivary glands by Marchand,[31] Ehrich,[16] Krompecher,[25] Masson and Peyron,[24a] Fraser and others.[26] It has found support recently in the histologic studies of Nieberle [2] on tumors of the mammary glands of dogs, in Tudhope's [24c] observations on a tumor of the human breast, in Sinard's [32] analysis of a tumor of the palm and in the investigations by Foulds [33] of a transplantable tumor of the oviduct of a domestic fowl. This concept enjoys the support also of Ewing.[11]

Three of the tumors described in this paper appear to point unequivocally to the transformation of epithelium first to the transitional stage of a myxomatous tissue and then to cartilage by the process of apparent inspissation and collagenization of a homogeneous *substance fondamentale* between the epithelial cells.

On this basis of epithelial derivation, the presence of carcinoma and of myxoid and chondroid tissue in the same tumor hardly need represent the chaos of a true organoid teratoma but a histologically comprehensible conversion of epithelium with definite transition stages. Nor is it surprising, therefore, that foci of cartilage are sometimes encapsulated, since—according to the interpretation herein advanced—they may arise from nests of glands which were originally surrounded by connective tissue septums. It is maintained that these are additional potent arguments against the theory of enclavement or of embryonic rests as applied to this type of so-called mixed tumor. The well known dermoids or teratomas, for instance, of the ovary, are considered examples of dysontogenesis and have no relation to this discussion.

EVIDENCE FROM EMBRYOLOGY OF THE NONSPECIFICITY OF GERM LAYERS

The thought of the mutation of germ layers is naturally somewhat repugnant to the adherents to the orthodox concept of *omnis cellula e cellula eiusdem generis*. They must reconcile, however, the development of cartilage from fetal ectoderm which is described by a number of investigators, including Platt [34] and Landacre and Warren.[35] From the field of experimental embryology, one learns further, through the ingenious investigations on the blastula and gastrula of amphibians, that if cells which were destined to form the epidermis of skin are transplanted to the orbital cavity they may differentiate into nerve, muscle, notochordal, cartilaginous or other cells (Bautzmann [36]; Kusche [37]). This emphasizes the realization that "the strictly preformistic conception of development has been losing ground. We have come to realize that development means the bringing into existence of new characters,

31. Marchand, F.: Verhandl. d. Gesellsch. deutsch. Naturforsch. u. Aerzte **82** (pt. 2): 1, 1910.
32. Sinard, L. C.: Am. J. Cancer **33**:182, 1938.
33. Foulds, L. A.: J. Path. & Bact. **44**:1, 1937.
34. Platt, J. B.: Anat. Anz. **8**:506, 1892.
35. Landacre, F. L., and Warren, J. H.: Anat. Rec. **14**:42, 1918.
36. Bautzmann, H.: Naturwissenschaften **17**:818, 1929.
37. Kusche, W.: Arch. f. Entwcklngsmechn. d. Organ. **120**:192, 1929.

not the bringing into appearance of pre-existing latent characters" (Weiss [38]).

Although tempting, one must gird oneself against the direct transfer of such evidence to the tissues—even the neoplastic tissues—of the fully developed organism, as Woglom [13] has indicated. Yet, it does seem to suggest that if a cell is sufficiently immature it possesses a plasticity which allows it to be molded by the multiplicity of physical, chemical and possibly other factors composing the environment. This subjugation of the cell to surrounding forces appears to be the master key to the problem of cellular variability, rather than a vitalistic inherent cellular endowment. The attempt will be made in this report to show that this environmental concept may be applied to the so-called mixed tumors (other than organoid teratomas), in which the cells appear to exhibit a versatility generally conveniently considered to be potentiated by specific endowments but for which a mechanistic explanation appears more tenable and more tangibly applicable to what one observes histologically.

"PSEUDOCARTILAGE"

It must be mentioned that many of the authors who describe the same type of transformation of epithelium as observed here are unwilling to concede that the resultant tissue is really cartilage. Accordingly, it is referred to or at least considered as pseudocartilage (for example, Fry,[23f] Tudhope [24e] and Budd and Breslin [39]). A similar interpretation is applied by many observers to the cartilage in the tumors of the salivary gland (for example, Marchand,[31] Leroux and Leroux-Robert,[40] Böttner,[41] Boyd [42] and Foulds [33] to cartilage in a tumor of the oviduct of a domestic fowl). On the other hand, although their views on the histogenesis may vary, the great majority of investigators nevertheless have expressed the belief that the tissue in both salivary and mammary tumors is true cartilage (for example, MacCallum [22g] and Ewing [11]).

If the concept of the epithelial origin of cartilage has merit, one should hardly rest on the mere histologic resemblance of the epithelial product to cartilage. Rather, one is logically required to assume the burden of matching each of the components of normal cartilage with a component of the "epithelial cartilage" and of tracing the latter from its epithelial progenitor.

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38. Weiss, P.: Physiol. Rev. **15**:639, 1935.

39. Budd, J. W., and Breslin, F. J.: Am. J. Cancer **31**:207, 1931.

40. Leroux, R., and Leroux-Robert, J.: Bull. Assoc. franç. p. l'étude du cancer **23**:304, 1934.

41. Böttner, O.: Beitr. z. path. Anat. u. z. allg. Path. **68**:364, 1921.

42. Boyd, W.: A Text-Book of Pathology, ed. 3, Philadelphia, Lea & Febiger, 1938, p. 768.

Chondrocytes.—It has not been shown that the cells in the epithelial cartilage differ in any essential detail of behavior or of morphologic appearance from chondrocytes. In both, distinctly basophilic capsules are present. In places the perilacunar basophilism is widened to a thick cuff, such as is seen commonly in ordinary cartilage. From one to four cells are found within the lacunae, and in an occasional lacuna the cell appears to have dropped out, just as may occur in a section of normal hyaline cartilage. The cells are more or less circular, although some irregularity in shape is present, which is generally ascribed to differences in pressure. The nuclei are for the most part vesicular and contain small nucleoli. Some of the nuclei, however, are pyknotic. Such pyknosis is not uncommon in normal growing cartilage and is attributed usually to poor penetration of the fixing fluid (Shipley [43]). Most strikingly characteristic are the large cytoplasmic vacuoles and the cytoplasmic strands which extend to the lacunar walls as if the remainder of the cell had shrunken. These vacuoles contain abundant globules of glycogen and small fat droplets. The amount of glycogen is pronounced and characteristic, too, of normal hyaline cartilage. In short, the essential features of the cells of normal hyaline cartilage are shared by the cells of the epithelial cartilage herein described.

Matrix.—It is maintained by others that the matrix of the "pseudocartilage" consists merely of a homogeneous mucus surrounding the epithelial cells (for example, Boyd [42] in reference to tumors of the salivary glands). As evidence against such an interpretation, one may mention three specific features common to the matrix of normal cartilage and the matrix of epithelial cartilage, which distinguish the latter from the mucoid intercellular fluid of the nonchondrified portions:

1. The extreme metachromasia with toluidine blue.

2. The progressively greater degree of carminophilia with increasing resemblance of the transitional stages to cartilage. It is indeed significant to note that this distribution of glycogen in the epithelial cartilage quite parallels the situation in the developing epiphysial cartilage. Here, too, the cells of the mature cartilage contain the most glycogen, there being a gradual diminution in the younger, transitional cells (Harris [19]).

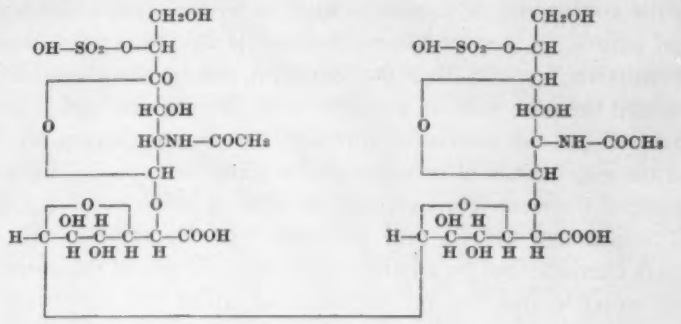
3. The transformation or replacement of the mucoid fluid by collagen.

So much for the general characteristics of the matrix. When one further analyzes the matrix, one finds much to indicate a close relationship between the "mother substance" and the chondral product, particularly from the chemical standpoint. Of course, one cannot state

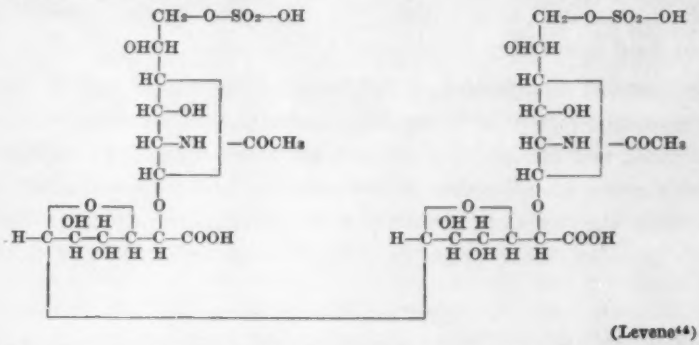
43. Shipley, P. G.: Cartilage and Bone, in Cowdry, E. V.: Special Cytology, New York, Paul B. Hoeber, 1928, vol. 2, pp. 703-733.

with certainty the exact chemical nature of the original fluid between the epithelial cells, nor that of the final product of the transformation. However, the former does possess an affinity for carmine and muci-carmine—variable and often slight, to be sure—and so it is generally assumed to be a mucoid fluid with mucoitin–sulfuric acid as its characteristic constituent. It is relevant, therefore, to indicate by formulas the intimate relationship between this compound and one of the basic organic constituents of the chondroid and osteoid matrix, namely, chondroitin-sulfuric acid:

Mucoitin–sulfuric acid:



Chondroitin–sulfuric acid:



(Levene[44])

Each compound contains one acetyl group to one molecule of glycuronic acid, sulfuric acid and a sugar. This sugar is chitosamine in mucoitin–sulfuric acid and chondrosamine in chondroitin–sulfuric acid. The difference lies principally in the fact that the sugars are isomers of each other. The position of the sulfur group is arbitrary and is assigned to indicate a difference in behavior toward hydrolytic agents (Levene [44]).

44. Levene, P. A.: Hexosamines: Their Derivatives and Mucins and Mucoids, Monograph 18, Rockefeller Institute for Medical Research, 1922.

This close relationship between mucin and the matrix of cartilage has been previously emphasized by Mathews [45] and recently by Téchoueyres.[46] In spite, however, of the fact that the chemical differences are slight, mucoitin has not been converted to chondroitin in vitro, and theoretically such a conversion would be most difficult (Sobotka [47]). There appear, therefore, to remain three possibilities for the development of a part of the matrix of cartilage from a mucoid fluid: (1) that the original intercellular fluid and subsequent matrix contain chondroitin rather than mucoitin (chondroitin–sulfuric acid is not confined to cartilage but is present in other locations [Levene [44]] not having the consistency of cartilage, such as tendon, sclera and aorta); (2) that nature, by some unknown method, is able to convert mucoitin into chondroitin, or (3) that the mucoitin retains its chemical composition and becomes part of a matrix with the physical and histologic characteristics of the matrix of cartilage. One must remember, after all, that the exoskeleton of invertebrates is composed of a polysaccharide (polymerized monacetylglucosamine) containing chitosamine and acetic acid as does mucin (Hawk and Bergheim [48]). Nevertheless, the exoskeleton is characterized by a hard consistency. It would therefore seem perfectly possible that the mucoid material about the neoplastic cells hardens similarly so as to form a matrix with a consistency resembling cartilage. It is of further interest that some varieties of mucin contain chondroitin–sulfuric acid (Wells [49]). In addition, the conversion of mucoid fluid to collagen is admitted by competent observers.[50]

Collagenous Component of Cartilage.—The second and by far the most abundant of the basic organic constituents of cartilage is collagen (Maximow and Bloom [51]). One is therefore obliged to account for the conversion of a portion of the mucoid fluid into collagen. It is not within the scope of this report to include the evidence for the various theories of the origin of collagen (see Doljanski and Roulet,[52]

45. Mathews, A. P.: Physiological Chemistry, ed. 5, New York, William Wood & Company, 1931, pp. 333-335.

46. Téchoueyres, E.: Ann. d'anat. path. **71**:905, 1934.

47. Sobotka, H.: Personal communication to the author.

48. Hawk, P. B., and Bergheim, O.: Practical Physiological Chemistry, ed. 10, Philadelphia, P. Blakiston's Son & Co., 1931, pp. 154 and 207.

49. Wells, H. G.: Chemical Pathology, ed. 2, Philadelphia, W. B. Saunders Company, 1914, p. 379.

50. Ehrich.[16] von Ebner.[17] Ricker and Schwalb.[18] Ewing.[11]

51. Maximow, A. A., and Bloom, W.: A Text-Book of Histology, ed. 3, Philadelphia, W. B. Saunders Company, 1938, chaps. 6 and 7.

52. Doljanski, L., and Roulet, F.: Virchows Arch. f. path. Anat. **291**:260, 1933.

Maximow,[53] Arey [54] and Hass [55] for review and bibliographies). It suffices to summarize the principal theories in the following condensed form:

1. The intracellular—according to which the fibers originate from (*a*) the homogeneous peripheral rim of ectoplasm; (*b*) the mitochondria or cytoplasm proper.

2. The intercellular theory—whereby the cells secrete an amorphous sol which is transformed by cellular enzymes into a gel with consequent condensation into fibers of collagen.

3. The direct transformation of fibrin (Baitsell [56]) or a *substance fondamentale* (Nageotte [57]) into collagen, a process in which fibroblasts are considered to play an entirely secondary role.

The last theory of the direct transformation of an amorphous or fibrinous substance through the action of cellular enzymes or possible mechanical factors has in recent years been receiving increasing attention, principally through the impetus of the observations of Baitsell,[56] Nageotte [57] and Doljanski and Roulet.[52] A direct application of this concept is made to clots, for example, where, it is maintained, the collagen is formed by the gelation of the fibrin of the exudate. Harrison,[58] after a careful study of the development of the amphibian balancer (rodlike props attached to the sides of the heads of larvae), came to the conclusion that collagenous fibers developed in an *acellular* mesenchymal matrix under the enzymatic influence of adjacent *epithelial* cells. As a result of their observations, Ehrich,[16] von Ebner,[17] Ricker and Schwalb [18] and Ewing [11] are convinced that mucus may be directly transformed into collagen. Finally, it is interesting to note that McKinney,[59] on the basis of studies of tissue cultures, concedes that "it is by no means proved that the fibroblasts are the only cells which are concerned in the formation of collagenous fibers." These details seem, then, to indicate convincingly the possibility of the transformation of mucoid, fibrinous or protein fluids into collagen, which, it is recalled, is one of the principal organic constituents of the matrix both of cartilage and bone. If this transformation becomes a universally recognized fact, a prominent barrier to the acceptance of the mechanistic conception of the conversion of such fluids into a *chondroid* matrix—no matter if the cells concerned are ectodermal or mesenchymal—will have been automatically removed.

53. Maximow, A. A.: Bindegewebe und blutbildende Gewebe, in von Möllendorff, W.: Handbuch der mikroskopischen Anatomie des Menschen, Berlin, Julius Springer, 1927, vol. 2, p. 549.

54. Arey, L. B.: Physiol. Rev. **16**:327, 1936.

55. Hass, G. M.: Arch. Path. **27**:583, 1939.

56. Baitsell, G.: Am. J. Physiol. **44**:109, 1917; J. Exper. Med. **21**:455, 1915; **23**:739, 1916; Am. Rev. Tuberc. **21**:593, 1930; Quart. J. Micr. Sc. **69**:571, 1924.

57. Nageotte, J.: Compt. rend. Soc. de biol. **79**:833 and 940, 1916; **82**:277, 1919.

(Footnotes continued on next page)

Now, then, the arguments in favor of the tissue within the tumors being "pseudocartilage" are said to be: (1) the frequent absence of perichondrium (Leroux and Leroux-Robert [40]); (2) the substitution of epithelial cells for chondroblasts (Tudhope [24e]; Foulds [23]; Leroux and Leroux-Robert [40]), and (3) the persistence of the mucus as matrix instead of a true chondroid matrix (Boyd [42]).

Brief deliberation on the role of the perichondrium in chondrogenesis will show why its presence or its absence is thoroughly irrelevant to the issue concerning "pseudocartilage." The perichondrium is normally a mesenchymal source for additional cartilage. Therefore, it is quite as if one were to say, in effect, that the "epithelial cartilage" is not cartilage because it does not arise from mesenchyme. Such an argument—based on the very premise which is being disputed, namely, that cartilage may arise only from mesenchymal tissue—obviously can hardly be used as evidence. Moreover, to label a tissue "pseudocartilage" simply because its cells are epithelial in origin is to avoid taking into account the facts of the presenting observations. As previously stated, these epithelial cells within the cartilage are similar to chondrocytes in all essential details. Finally, evidence showing that the mucus does not persist as such but becomes converted into a matrix indistinguishable from chondral matrix has been presented in the preceding paragraphs.

In summary, it would therefore seem that under certain conditions strikingly present in the canine mammary tumors (for instance, trauma, inflammation, necrosis, hemorrhage, retiform cellular pattern) epithelial cells of the adult type derived from ectoderm in combination with their interstitial substance may become transformed into a tissue indistinguishable from cartilage. The requisites appear to be physicochemical and include a cell—be it fibroblastic, chondroblastic or indeed epithelial—arranged in a more or less reticular pattern or otherwise scattered in a matrix. It is entirely conceivable, and consistent with the evidence herein advanced, that this same general mechanism is the basis of normal chondrification as well as ossification. This mechanistic interpretation is the central thought of this report and will be further elaborated under "Comment."

CHONDRIFICATION IN COLLOID CARCINOMA OF THE HUMAN BREAST

The question that logically arises next is why chondrification does not occur in the colloid (gelatinous or mucous) carcinoma of the human breast, inasmuch as an apparently chondrifiable milieu is present. One may wonder whether or not the mucoid fluid of the canine breast differs chemically from that of the human breast, since mucins in other parts of the body are known to differ from each other in chemical composition

58. Harrison, R. G.: J. Exper. Zoöl. **41**:349, 1925.
59. McKinney, R. L.: Arch. f. exper. Zellforsch. **9**:14, 1929.

and properties (Levene [44]). There are no known data on this point, and it must therefore remain moot for the present. However, a significant detail concerning this gelatinous fluid is the fact that it does occasionally manifest an affinity for calcium (Shore [60]). This affinity is demonstrable in one of the cases included in this report and tends to suggest that the reason for the failure to chondrify lies possibly not with the mucin but with the viability and arrangement of the cells.

Polarity.—The question of the arrangement of the epithelial cells in these and other colloid carcinomas is of great interest. The phenomenon is apparently dependent on cellular polarity. Masson,[24b] in his investigations of mixed tumors of the parotid gland, laid much stress on this feature of cellular function. The polarity of the cell apparently determines whether a group of cells will assume an acinar, nestlike, fascicular or reticular pattern. For instance, if the cell possesses an interstitial or diffuse polarity, its secreting surface is diffuse instead of at one side. The obvious result of such a diffuse secreting surface is that the fluid accumulates on all sides of neighboring cells, which are consequently forced apart and assume first a spongy, and then a myxomatous, pattern. One immediately conceives of a direct application of this to the problem of the canine tumors and the human tumors of the colloid variety. It will be recalled that the colloid carcinomas were composed not of reticulated epithelial cells but of solid clumps which showed practically no evidence of intercellular accumulation of fluid (fig. 11). The fluid appeared rather to surround the nests of epithelium. An application of Masson's scheme seems to indicate that there is a difference in polarity between the clumps of epithelial cells of the colloid carcinoma of the human breast as opposed to the cells in the canine mammary tumor. At any rate, the difference in arrangement of the cells is regarded of major importance in the failure of chondrification of the colloid not only in the human breast but also in the colloid or mucous carcinomas of other organs, for example, the gastroenteric tract and the ovary. The cells within the alveoli or cysts of these tumors are generally cast-off, degenerating cells or at least cells which are destined soon to degenerate. As a matter of fact, Geschickter [61] expressed the belief that the mucus formed in the colloid carcinoma of the human breast is related actually to interference with the blood supply.

It is maintained that by the very nature of their generally more or less retiform pattern of growth, as opposed to the solid or alveolar pattern of carcinoma, the sarcoma—in quite the same way as proliferating fibroblasts—lends itself much more easily to chondrification as well as ossification because of the ease with which such cells are separated by the transuded or secreted fluid or by a collagenous tissue

60. Shore, B. R.: Am. J. Cancer **15**:221, 1931.
61. Geschickter, C. F.: Ann. Surg. **108**:321, 1938.

undergoing mucoid change. It must be borne in mind, after all, that soft tissue sarcomas do form bone not altogether infrequently. It is only when the epithelial cells—even epithelial cells of the adult type—simulate this arrangement, as in the canine mammary tumors, the mixed tumors of the salivary glands and other regions, and possibly in tumors of the type described by Woglom,[13] that the stage for chondrification is set. This pattern, independent of inherent cellular potencies of a specific nature, appears to me to be the key to the phenomenon of "epithelial chondrification." An illustration of this general principle is afforded by the transplantable mammary carcinoma of mice studied by Woglom.[13] In the second generation the cells became spindle shaped. Sarcomatous transformation was thought to have occurred, and, indeed, cartilage was found in 3 of the 4 tumors of this generation. The very presence of cartilage was considered strong evidence that the tumor had become sarcomatous. However, one must reckon with the fact that cells of epithelial tumors (for example, adamantinoma, thyroid carcinoma, basal cell carcinoma) may acquire a spindle shape (Ewing[62]) and that even in cultures of epithelial cells it may be impossible to distinguish some of the cells from fibroblasts.[63] As a matter of fact, this same transplantable carcinoma of mice which was regarded as having undergone sarcomatous change was considered by Ewing[62] to be actually a spindle cell form of carcinoma. Slye, Holmes and Wells[12] described a somewhat similar spontaneous tumor of a mouse. One does not mean to capitalize on these differences in interpretation of an exceedingly difficult problem; they are mentioned merely because they at least tend to bear out the possibility that the proliferating cell taking part in the process of chondrification may, on occasions, be ectodermal as well as mesenchymal.

REASONS FOR DISPARITY IN INCIDENCE OF CARTILAGE AND BONE IN CANINE AND HUMAN MAMMARY TUMORS

Closely related to the questions of histogenesis is the second phase of the problem, which concerns itself with the reasons for the disparity in incidence of cartilage and bone-containing tumors of the mammary glands of dogs and man. Peyron[64] maintained that the increased incidence in dogs is directly attributable to the abundance of myoepithelial cells in this species as contrasted with man. This cannot be confirmed. Glendining[15] suggested that there is an "apparent interchangeability of mesoblastic structures" of the dog because they are less highly specialized than in man. If Glendining meant to say that the stromal tissue of the canine mammary gland is not as mature or possesses a greater lability than the corresponding tissue of the human breast, he must bear the burden of a proof which has not been demonstrated.

62. Ewing, J.: J. Cancer Research **1**:71, 1916. Ewing.[11]
63. Nordmann, M.: Arch. f. exper. Zellforsch. **9**:54, 1929.
64. Peyron, M. A.: Bull. Assoc. franç. p. l'étude du cancer **13**:349, 1924.

The following factors suggest themselves as having a possible bearing on the question:

1. The acknowledged tendency toward sarcomatous change in the mammary glands of dogs as contrasted with man.

2. The greater liability of the canine mammary glands to trauma, inflammation, hemorrhage and necrosis.

3. A possible species difference in bone-forming propensity.

1. *Sarcomatous Tendency.*—The well recognized tendency for sarcomatous change to take place in the mammary gland of the dog as contrasted with man is considered of importance in the production of cartilage and bone not because of any inherent specific potency in that direction but for the following two reasons:

(*a*) As stated, from the mechanistic point of view the sarcomatous structure is suited for the formation of cartilage and bone by virtue of its retiform pattern and the consequent ease of separation of cells when the fluid is secreted or transuded.

(*b*) Sarcomas are exceedingly prone to necrosis, hemorrhage and inflammation—factors which are held to be of significance in the general phenomenon of the formation of cartilage and bone. This is to be discussed in more detail in the following paragraphs.

2. *Trauma, Necrosis, Hemorrhage and Inflammation.*—The role of trauma in the chondrification and ossification of tumors of the canine breast merits consideration. It is a fact that these tumors occur predominantly in the hindmost breast (Nieberle [2]). This is the largest of the glands, and during lactation it secretes the most milk and becomes the most pendulous. The subcutaneous tissue is loosest in this region, which permits the gland of the active animal to swing freely and thereby to subject itself to repeated trauma and strain.

The possible role of trauma in the proliferation and tumefaction of cartilage and bone has been appreciated for a long time. It is of course thoroughly appreciated how difficult it may be to evaluate an etiologic agent of such a character. This and related points were justifiably emphasized by Ewing.[65] Behan,[66] who recently reviewed the subject comprehensively, included an extensive bibliography of observers who favor the thesis that trauma has a distinct bearing on the initiation and further development of tumors of the skeleton. On the other hand, a not inconsiderable number were cited who discount any such relationship. Virchow [1a] strongly emphasized the factor of trauma in the causation of enchondroma and pointed out that the sites of these tumors were the sites of liability to trauma. Modern statistics confirm the relationship between the skeletogenous tumor and the specific history of definite

65. Ewing, J.: Arch. Path. **19**:690, 1935.

66. Behan, R. J.: The Relationship of Trauma to New Growths, Baltimore, Williams & Wilkins Company, 1939.

trauma (Geschickter and Copeland [67]; Kolodny [68]). The relation of mechanical factors, such as trauma, stress and strain, to chondrification and ossification has been repeatedly demonstrated also in vitro (for instance, Glücksmann [69]).

Even if trauma were to be regarded as an exciting factor in the proliferation of cartilage and bone, it would be appreciated that the objection might be raised that skeletogenous tumors may not be strictly comparable to soft tissue tumors containing cartilage and bone. The difference lies really in the assumption that in the former there are present tissues which are directly chondrogenic or osteogenic, whereas in the latter such tissues are lacking. However, this assumed difference is hardly the fact inasmuch as cells of soft tissue sarcomas, and even epithelium under certain circumstances, may become chondrogenic or osteogenic (Mallory [70]). This potentiality, it need hardly be mentioned, is not nearly as marked as it is in the periosteum or perichondrium, and by the same token the incidence of bony and cartilaginous tumors is not nearly as great in the soft parts as in the skeleton. The point is that this difference in incidence does not preclude a general factor or combination of factors, such as trauma, stress, strain, hemorrhage, necrosis and inflammation, as part of the setup which initiates chondrification and ossification. That a metaplasia of connective tissue into cartilage may take place under the influence of mechanical factors can hardly be doubted, according to Maximow and Bloom.[51]

It must be stated clearly that the issue of present concern is not related to the question of the relation of trauma to the initiation of carcinoma or sarcoma but simply to the matter of cartilage and bone production. Moreover, it is desired emphatically to avoid the implication that trauma per se—that is, the mere application of force—is responsible for chondrification or ossification. The important features of trauma in this regard are its sequelae, namely, interference with circulation, hemorrhage, necrosis, edema and inflammation. It is not difficult to understand that inflammation with edema, necrosis, ulceration and hemorrhage within the tissues occurs frequently in sites so exposed and subjected to repeated trauma as are the tumors of the hindmost canine mammary glands. Contrasted with the canine tumor, the human mammary carcinoma is, as a rule, relatively small in proportion to the size of the breast and is cushioned by much fat. The canine tumor, on the other hand, is generally exposed to the surface or covered merely by a thin capsule and skin (fig. 7) so that the impact of trauma to the region is borne by the neoplasm. Inflammation, with hemorrhage

67. Geschickter, C. F., and Copeland, M. M.: Tumors of Bone, New York, American Journal of Cancer, 1931.

68. Kolodny, A.: Surg., Gynec. & Obst., 1927, supp. 1, p. 1.

69. Glücksmann, A.: Anat. Rec. **73**:33, 1939.

70. Mallory, T. B.: Am. J. Path. (supp.) **9**:765, 1933.

and necrosis, was present in the 4 canine growths and in the partially chondrified human tumor included here. The large size and rapidly growing sarcomatous nature of the latter neoplasm predisposed it toward hemorrhage, necrosis and inflammation. These factors, it is maintained, are of major importance in the tendency toward calcification, chondrification and ossification of the mammary gland of the dog in the same way as they are considered to play the predominant role in the calcification and ossification of a variety of other organs subjected to these influences.[71] In this connection, one should like to make the point that the parotid region suffers a great deal more constantly repeated trauma by the simple act of mastication than is generally appreciated. One can evaluate the degree of this trauma roughly by palpation of the masseter muscles with the teeth clenched as in chewing. Indeed, about a quarter of a century ago, Fraser[26] postulated that trauma was an important factor in the origin of mixed tumors of the salivary glands.

3. *Experimental Ossification Under the Influence of Epithelium.*—From the field of experimental surgery, one learns the interesting fact that when fascia is transplanted to an artificial defect in the urinary bladder of a dog bone forms within the fascia (Neuhof[72]). Moreover, transplantation of a strip of epithelium from the urinary bladder of a dog to the rectus fascia constantly causes bone to be formed directly adjacent to the border of the transplanted epithelium (Huggins;[73] Abbott, Goodwin and Stephenson[74]). The same experiment in rabbits produced bone in only 1 of 6 animals.[73] The administration of large doses of viosterol and parathyroid to such experimental rabbits failed to stimulate ossification at the sites of the transplanted epithelium. Connective tissue transplants to the urinary bladders of rabbits produced no bone in contrast to the results in dogs (Phemister[75]). Ossification was observed also in guinea pigs and rats by Huggins, McCarroll and Blocksom,[76] but to a decidedly lesser extent by Abbott, Goodwin and Stephenson[74] in their guinea pigs and cats. On the other hand, Gruber[71b] found it difficult to produce traumatic myositis ossificans in dogs as contrasted with rabbits.

It is further worth noting that the epithelial transplants to the fasciae of the dogs formed cysts containing usually about 2 to 4 cc. of fluid,

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71. (*a*) Harvey, W. H.: J. M. Research **12**:25, 1907. (*b*) Gruber, G. B.: Ueber Histologie und Pathogenese der circumskripten Muskelverknocherung, Jena, Gustav Fischer, 1913. (*c*) Sacerdotti, C., and Frattin, G.: Virchows Arch. f. path. Anat. **168**:431, 1902. (*d*) Virchow.[1a] (*e*) Bunting.[29b] (*f*) Leriche and Policard.[29h]

72. Neuhof, H.: Surg., Gynec. & Obst. **24**:383, 1917.

73. Huggins, C.: Arch. Surg. **22**:377, 1931.

74. Abbott, A. C.; Goodwin, A. M., and Stephenson, E.: J. Urol. **40**:294, 1938.

75. Phemister, D. B.: Ann. Surg. **78**:239, 1923.

76. Huggins, C. B.; McCarroll, H. R., and Blocksom, B. H.: Arch. Surg. **32**: 915, 1936.

which was examined for calcium, phosphorus and hydrogen ion concentration.[73] The values for calcium and phosphorus in the cysts were strikingly higher than those for these elements in the blood, and the p_H was distinctly lower. On the other hand, the values of phosphorus and of p_H for the corresponding 3 rabbits quite matched those of the blood. (Calcium values were not given.) In other words, these data indicate a fundamental mensurable difference in the ossifying ability of at least the dog and rabbit and imply a significant role of the epithelium in the production of bone under these conditions. As to what the corresponding results in the human subject would be, there are unfortunately no data. If such data were available, one might then, it is not unlikely, possess an additional clue to the problem of the disparity in the incidence of cartilage and bone in tumors of the mammary glands of dogs and of man on the basis of species differences. Chemical examination of the mucoid material of both human and canine breasts not only for glycoproteins but for calcium, phosphorus, phosphatase and p_H might furnish additional significant data.

Of course, if there is in fact a specific tendency toward ossification in dogs, one might reasonably expect evidence thereof in tumors other than those of the mammary glands. And to be sure, according to Ruddock,[77] ossification in malignant growths of the thyroid occurs most frequently in dogs. However, in view of the high incidence of malignant changes of the thyroid in these animals (Slye, Holmes and Wells[78]) this observation might best be subjected to a statistical analysis.

COMMENT

1. The observation of the development of cartilage from *adult* epithelium of the mammary gland of the dog is the pivot about which most of this discussion turns. This observation would constitute a mere academic minutia if one were to concern oneself merely with the mammary gland. However, to state in effect that cartilage, a mesenchymal derivative, may be formed directly from an adult ectodermal derivative, or that the chondrocyte may be replaced by an epithelial cell, is to challenge the orthodox concept of the specificity of germ layers—a concept which forms the basis of present day teaching of many phases of biology. Adherents to this concept might explain this observation in one of two ways:

(*a*) That it represents a vitalistic monstrosity, with anaplasia of the epithelium to immature, multipotent cells, followed by differentiation into cartilage.

(*b*) That the tissue is not true cartilage.

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77. Ruddock, H. B., and Willis, R. A.: Am. J. Cancer **33**:205, 1938.

78. Slye, M.; Holmes, H. F., and Wells, H. G.: J. Cancer Research **10**:175, 1926.

In the text of this report, many reasons have been advanced to indicate the inadequacy of the explanations offered by this conveniently flexible concept of the vitalists, who must necessarily meet problems of this sort merely by turning the clock backward or forward or, if it suits the occasion, in both directions. One is not justified in assuming that the mere anaplasia of the cells of an adult organism, even though accompanied by the properties of rapidity of growth and invasiveness, *ever* carries with it the other potentialities of fetal cells. More decisive, however, is the fact that the epithelium concerned in this chondrification is, histologically at least, of the adult type—being portions of obviously mature mammary acinar epithelium. In answer to the second, more serious objection it is stated that this epithelial cartilage has been matched component for component with normal cartilage as far as known morphologic, biochemical (pancreatic digestion) and tinctorial criteria permit, and found indistinguishable therefrom.

It is maintained that the development of this cartilage from adult epithelium is the result of environmental influences, and, to be sure, the observations of the transitional stages comply neatly with a mechanistic explanation. This interpretation of the chondrification of the epithelium is compatible with the "newer" embryology, which attempts to eliminate the preformistic concept of cellular endowments and attributes the characteristics of a cell to the dictates of its environment (Weiss [38]). This is vividly illustrated by the development of a blastulous cell known ordinarily to form epidermis of skin, which when transplanted may develop into muscle, notochordal, cartilaginous or other cells. In other words, it is not meant to imply that if the individual cells of the epithelial cartilage were transplanted to tissue cultures they would necessarily form cartilage, for the reason that the environmental factors would be changed and might therefore not be suitable.

2. The second ramification of this basic phenomenon concerns the matrix. The intercellular fluid, originally of a mucoid nature, becomes transformed into or replaced by a collagenized matrix indistinguishable from a chondral matrix. Is this a true transformation of mucin directly into collagen—a phenomenon acknowledged by several prominent observers (Ewing;[11] Ehrich;[16] von Ebner;[17] Ricker and Schwalb [18])—or has the mucin been replaced by collagen derived from the surrounding connective tissue, possibly with the participation of the mucin? As stated, the chemical relationship of mucoitin to chondroitin is close, just as the mucin and the final chondral matrix appear to be morphologically in these tumors. However, an accurate positive statement as to which of these two possibilities is correct is obviously impossible, although it appears not unlikely that both the intercellular mucoid fluid and the preexisting collagen take part in the formation of the final matrix.

3. A more immediate question concerns the interpretation of the heterogeneity of the tissues in these tumors or, in other words, the term "mixed tumor." In one tumor (dog 166) the bone and part of the cartilage were formed by transformation of the stromal tissue. Inasmuch as such ossification and chondrification, even though extensive, as in this case, are really simply heterotopic stromal formations, this type of tumor would more properly be referred to as a carcinoma or a sarcoma with stromal ossification or chondrification, rather than as a mixed tumor. In those neoplasms in which the tumor cells themselves take part in the formation of bone or cartilage, the qualifying terms "myxo-," "chondro-," "osteoid-" or "osteo-" should be used to modify "carcinoma" or "sarcoma." For example, the diagnosis myxochondroadenocarcinoma should imply that the myxomatous and cartilaginous cells are epithelial in origin (as, for example, in dog 322). Similarly, the term "osteosarcoma" should be applied to corresponding soft tissue tumors. The diagnosis osteo*genic* sarcoma, as Kolodny [68] suggested, ought then to be restricted to tumors arising directly from the skeleton.

4. In one tumor (dog 166) small foci of cartilage were observed forming nearby large bony trabeculae. The process seemed to be not an ossification of cartilage but an independent formation of each from adjacent portions of the same tissue, that is, the stroma. This is confirmatory evidence for the more or less generally accepted belief that the cells taking part in the formation of cartilage may be of the same type as those participating in ossification. More direct evidence is the observation of ossification of cells of the perichondrium, on the one hand, and of chondrification of periosteal cells, on the other. This may be observed in vitro (Glücksmann [69]).

5. The development of fibrocartilage from the mature, sclerotic collagenous tissue of the fibromyxochondrosarcoma of the human breast raises an additional fundamental question. As stated, one observes the transformation of the collagenous tissue into fibrocartilage quite as if the cartilaginous formation occurred after the adult collagenous tissue had been formed. Generally fibrocartilage is assumed to develop from immature mesenchymal cells with a particular potency for chondrification. The precise environmental factors responsible for this aberration are unknown at present, although it is probable that they, too, are stress, strain, inflammation and necrosis. Such a phenomenon, as well as the production of cartilage from epithelium, adds to the accumulating evidence against the specificity of chondroblasts.

6. Closely related to the question of the direct transformation of collagenous tissue to fibrocartilage is that of heterotopic ossification. The significance of the very familiar picture observed in foci of stromal ossification ought to be reconsidered in the light of certain histologic observations. One notes a transition between the ossified matrix and

the adjacent mature collagen of the stroma (figs. 8 and 9). One can actually observe the collagenous fibers from the surrounding stroma continuing on into the bony trabecula. The process seems to consist first of a homogenization of the collagen, due perhaps to necrosis, hemorrhage, inflammation and other processes. This homogenized collagen possesses an affinity for calcium. The cells of the original collagen—the fibrocytes—are imprisoned by the matrix and in this situation are called osteocytes. The result of this transformation is a bony trabecula, in which transformation the role of the cells appears to be of entirely secondary importance.

These same figures illustrate a second significant point. At *A* (fig. 8) one observes the line of advancing ossification. At this site, however, one notes the practical absence of cells that might be called osteoblasts. This is especially striking in contrast with the other surfaces of the same trabecula. If osteoblasts were responsible for this progressive ossification, one would logically expect to see them active at the line of advancing ossification. It would seem, therefore, that the new bony matrix in this type of ossification is derived not from osteoblasts but from a direct transformation of nonspecific collagen with nonspecific fibrocytes.

This observation and interpretation are not new. Similar examples are found classically in ossified tuberculous complexes. Weidenreich [79] mentioned several investigators other than himself who had observed the same type of picture and had applied a similar interpretation. Direct opposition to the vitalistic concept of the specificity of the osteoblast has been voiced in clearcut terms by Leriche and Policard,[29h] Grieg [80] and Murray.[81] T. B. Mallory [70] concluded from his observations of ossified soft tissue sarcomas that any fibroblast may take over the function of the osteoblast, a view stated in 1914 by F. B. Mallory.[82]

However, this mechanistic concept of heterotopic bone formation continues to meet with the resistance of the adherents to the traditional concept of intramembranous ossification as given in textbooks. For example, Ham [83] stated in Cowdry's "Special Cytology" that "there is no evidence to show that metaplastic bone develops by a metamorphosis

79. Weidenreich, F.: Das Knochengewebe, in von Möllendorff, W.: Handbuch der mikroskopischen Anatomie des Menschen, Berlin, Julius Springer, 1930, vol. 2, pt. 2, p. 435.

80. Grieg, D. M.: Clinical Observations on the Surgical Pathology of Bone, London, Oliver & Boyd, 1931, chap. 1.

81. Murray, C. R.: J. Dent. Research **11**:837, 1931.

82. Mallory, F. B.: The Principles of Pathologic Histology, Philadelphia, W. B. Saunders Company, 1914, pp. 275-277.

83. Ham, A. W., in Cowdry, E. V.: Special Cytology, ed. 2, New York, Paul B. Hoeber, Inc., 1932, vol. 2, p. 990.

of adult tissues." Rhode,[84] in an extensive article, emphatically denied the possibility of ossification by direct transformation of connective tissue and insisted that bone is invariably formed from osteoblasts or undifferentiated, "unused remaining mesenchymal cells." These osteoblasts are assumed to secrete or to become peripherally transformed into matrix (Shipley[43]; Maximow and Bloom[51]). Any increase in size of the trabecula is considered to be due to the activity of the osteoblasts.

However, there is yet a second familiar important morphologic observation, in addition to that of the direct transformation of collagen to bony matrix, which is explained inadequately by the standard concept, namely, the regular alinement of osteoblasts against the surfaces of a trabecula (fig. 8). This commonplace finding is generally regarded as a reflection of a behavior characteristically peculiar to osteoblasts and in a measure indicative of their specificity. Maximow and Bloom[51] stated that the increase in size of the trabecula is due to the secretory activity of the osteoblasts. They appear not to have accounted for the alinement other than to state that "the osteoblasts gradually move to the exterior." Does it not seem likely, on the one hand, if individual cells were secreting their varying amounts of matrix, that the trabecular surface would tend to be distinctly irregular and the cells irregularly alined? On the other hand, does it not seem more reasonable that the interstitial substance is originally of a fluid nature, derived perhaps from tissue fluids, lymph or plasma, and that the alinement is a compression phenomenon simulating in a sense the alinement of crumbs by a bread crumber or of debris on the beach by the incoming waves?

Regarding the origin of the interstitial substance from plasma, one is reminded of the hyalinoid material of the *Trummerfeld* zone and *Gerustmark* in scurvy, which is generally considered to be formed in part by the osteoid secretion of osteoblasts (for instance, Wolbach and Howe[85]) but which Aschoff and Koch[86] regarded fibrinous in nature. The presence of blood pigment in the *Gerustmark*[86] and the hemorrhagic tendency in this disease fortify the suggestion of the intravascular origin of this material. This is of utmost significance, because this hyalinoid material becomes transformed into the matrix of osteoid tissue.

It is relevant here to refer again to the fundamental observations of Baitsell, Harrison, Nageotte and Doljanski and Roulet on the development of collagen from plasma or serous fluids with no apparent or only an indirect influence of cells. These observations furnish the basis for the formulation of a theory of intramembranous ossification which seems to be more directly in accord with the morphologic facts and which

84. Rhode, C.: Surg., Gynec. & Obst. **41**:740, 1925.

85. Wolbach, S. B., and Howe, P. R.: Arch. Path. **1**:1, 1926.

86. Aschoff, L., and Koch, W.: Skorbut: Eine pathologisch-anatomische Studie, Jena, Gustav Fischer, 1919.

does not circumvent the observations of heterotopic ossification by the use of vitalistic hypotheses based on ubiquitous mesenchymal cells of a specific nature.

The formation of collagen directly by the gelation of the colloidal sols of plasma serum or protein tissue fluids is considered to supply the foundation for the development of the matrix of osteoid tissue, of which collagen is the principal organic component. This fluid or, later, semisolid matrix alines the cells in the vicinity in the characteristic osteoblastic rim by virtue of its lateral pressure against them. If too few cells are present, naturally no such rim is formed; if the bone is developing adjacent to or in the midst of young, cellular, easily compressible granulation tissue, obviously the *likelihood of such an alinement is increased to the degree of the cellularity and compressibility.* The addition of further increments of interstitial substance to the newly developing trabecula appears to be responsible for the lamellations. With the increase in matrix, many of the osteoblasts are forced aside in a regular line by the wave of new material; others are imprisoned as osteocytes. This substance becomes further elaborated into osteoid matrix, and finally calcification occurs.

The evidence is not convincing by any means that participation even in the biochemical processes concerned with calcification is a specific property of osteoblasts. It must be recalled, for example, that in the kidneys of certain animals the phosphatase activity is fully 50 per cent of that of an equal weight of epiphysial cartilage (Robinson [87]). An interesting experiment illustrating the immediate unimportance of living cells in the process of calcification of cartilage under certain conditions is furnished by Wells and Benson.[88] They heated pieces of epiphysial, rib and tracheal cartilage to a temperature judged sufficient to kill the cells and placed the pieces in the peritoneal cavity for a varying number of weeks. The calcification of the epiphysial cartilage was found to be strikingly greater than that of either the rib or the tracheal cartilage. In other words, that cartilage which would have normally ossified in situ became calcified in the peritoneal cavity, whereas the other cartilages —costal and tracheal—which normally do not ossify in situ, showed relatively little calcification. To repeat, this phenomenon occurred in the absence of living cells or of "vital action" [88] within the cartilages.

The phenomenon of heterotopic ossification dovetails easily into the concept outlined, which stresses, first, the extracellular development of both collagen and osteoid matrix and, second, the close relationship of collagen to osteoid matrix and of both to a common interstitial substance. The collagen of the involved connective tissue is changed from its neighboring collagen by a variety of possible factors, such as trauma,

87. Robinson, R.: Biochem. J. **17**:286, 1923.

88. Wells, H. G., and Benson, R. L.: J. M. Research **12**:15, 1907.

stress, strain, necrosis and inflammation, with resulting homogenization and acquisition of an affinity for calcium.

SUMMARY

Four so-called mixed tumors from mammary glands of dogs and one from a human breast are described. These tumors contain myxomatous tissue, cartilage, osteoid tissue and bone. The tumor of the human breast is a fibromyxochondrosarcoma. Three of the canine tumors are carcinomas; the fourth is a sarcoma.

Contrary to the generally held concepts of the specificity of germ layers, the cartilage in three of the canine neoplasms appears to be *derived directly from adult epithelium.* The transition stages of the conversion of the epithelium into cartilage are easily followed and consist of (1) loosening of the acinar epithelium, (2) isolation of the epithelial cells in a matrix so as to simulate myxomatous tissue, (3) collagenization of the mucoid matrix and (4) homogenization and lacunar formation.

This "epithelial cartilage" is considered to be true cartilage as judged by known morphologic, biochemical (pancreatic digestion) and tinctorial criteria.

The development of fibrocartilage from mature, sclerotic collagenous tissue rather than directly from immature mesenchymal cells has been observed and its significance indicated.

A mechanistic theory of intramembranous ossification is outlined which accounts for both normal and heterotopic ossification without the use of the traditional vitalistic hypothesis of specific osteoblasts and ubiquitous undifferentiated mesenchymal cells.

Reasons favoring the nonspecificity of chondroblasts and osteoblasts are presented, with emphasis on the importance of environmental factors, in agreement with the "newer," mechanistic embryology.

The term "mixed tumor" as applied to this type of neoplasm is misleading and, if used at all, should be reserved for tumors composed of dysontogenetic tissues, such as organoid teratomas. "Myxo-," "chondro-," "osteoid-" or "osteo-" should be added to "carcinoma" or "sarcoma" to designate the type of tumor described here when the myxo, chondro, osteoid or osseous tissue is a direct modification of the tumor tissue. The presence of heterotopic stromal chondrification or ossification should of course not be indicated by qualifying adjectives.

Considerations similar to the foregoing are obviously applicable to other tumors of this type—for example, the so-called mixed tumors of the salivary glands.

Reasons for the strikingly high incidence of cartilage and bone in tumors of the mammary glands of dogs as contrasted with man are given. These are based on the liability of the former to trauma, with resulting necrosis, hemorrhage, inflammation and edema, and on the retiform pattern of the canine acinar epithelium.

EVALUATION OF THE APPARENTLY INCREASED INCIDENCE OF PRIMARY CARCINOMA OF THE LUNG

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Prior to 1900 primary carcinoma of the lung was believed to be exceedingly rare, and when a tumor was found in the lung it was almost always considered to be metastatic. Now carcinoma of the lung is thought to be quite common, ranking with carcinoma of the stomach, breast and uterus in frequency. Although the evidence suggests strongly that there have been both a relative and an actual increase in primary carcinoma of the lung, physicians should investigate carefully other possibilities before accepting this as a proved fact.

In any attempt to conform with changing ideas of tumor origin, it is natural that certain pendulum-like tendencies should develop. It is my purpose in this paper to suggest that such may be the case in the diagnosis of primary carcinoma of the lung.

It would be interesting in this connection to study the incidence of endothelioma of the pleura over a number of years. Unfortunately information on the incidence of this tumor is not readily available. Certainly the diagnosis was relatively common a few years ago, while now most of the cases are thought to be instances of primary carcinoma of the lung. The old "oat cell" tumor of the mediastinum, so commonly mentioned in English writings, is now thought by most pathologists to be primary in the lungs rather than in the lymph nodes. And much more recently the Pancoast tumor of the superior pulmonary sulcus appears to be slipping into the classification of primary carcinoma of the lung.

Most publications on the subject of primary carcinoma of the lung stress the multiplicity of clinical and histologic types. This in itself is enough to give one pause. Furthermore, the gross criteria are inexact. In most instances the tumor is thought to be primary in the bronchi, and involvement of the bronchial mucosa is a helpful diagnostic point at autopsy. This is not an adequate criterion, however, as will be shown later; in a good many instances a tumor primary elsewhere metastasizes to the bronchi. Some writers state that primary carcinoma of the lung

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may be manifest as bilateral multiple discrete tumor masses. This would seem to let down the bars to all sorts of tumors, primary elsewhere and metastatic in the lungs. I have recently seen a case of this sort, with multiple discrete tumors of about equal size in both lungs, diagnosed as one of primary carcinoma of the lung, although the autopsy had been restricted. The operator was a surgeon who rarely did an autopsy, and he stated that the pancreas was indurated and the liver contained nodules. No sections were obtained from the pancreas.

Even in the most experienced hands a primary tumor can be easily overlooked, especially if located in the nasopharynx. Furthermore, many cases of carcinoma show, at the time of necropsy, large masses throughout the body, any one of which could have been primary. It would appear that these could be easily differentiated on the basis of the cellular picture, but in many instances that is not the case. It is the poorly differentiated adenocarcinoma that is apt to give most difficulty in this regard and in such cases the tissue of origin may never be determined certainly. The direction and character of the metastases are frequently of help in arriving at a conclusion.

A case in point will illustrate the difficulties that may be encountered.

CASE 1

An emaciated 70 year old white man was admitted to the Gallinger Municipal Hospital in March 1939, with a history of difficulty in swallowing and breathing. There was a brief note on the hospital record that a tumor had been removed from the "throat" at another hospital in March 1937. There was a mass about 3 cm. in diameter just above the middle of the right clavicle. Physical examination revealed nothing else remarkable. The difficulty in swallowing became more severe, and a gastrostomy was done under local anesthesia. The man died ten days later, apparently of pneumonia.

Autopsy disclosed an abscess 10 cm. in diameter in the anterior portion of the lower lobe of the left lung, near the apex of the heart. A bronchus 8 mm. in diameter, leading into that portion of the lung which contained the abscess, was almost occluded by a soft intrabronchial tumor growth (fig. 1). The whole growth in this area measured 1.5 cm. in diameter. Several smaller nodules of tumor tissue were located near the bronchial growth, and flat umbilicated nodules were noted on the pleural surfaces of both lungs. Two lymph nodes in the hilus were greatly enlarged and contained tumor tissue. The mass in the right side of the neck consisted of neoplasm in lymph nodes there, intimately surrounding the right vagus and accessory nerves and infiltrating their sheaths. The jugular vein in the same area was completely obliterated by tumor growth. The larynx, esophagus and trachea showed no evidence of ulceration, tumefaction, scarring or other abnormality.

This case at the time of autopsy was thought to be one of primary bronchogenic carcinoma with metastasis to the mediastinal and cervical lymph nodes. The involvement of the vagus and accessory nerves was thought to have caused the dysphagia, which was the most conspicuous

clinical symptom in the case. The stenosing bronchial tumor, associated with a mediastinal metastasis and with a pulmonary abscess, appeared typical of primary carcinoma of the bronchus. Histologically, the tumor was composed of rather undifferentiated cells, obviously of squamous type; this is, of course, a common structure for primary carcinoma of the bronchus. On reviewing the case, however, I was struck by the fact

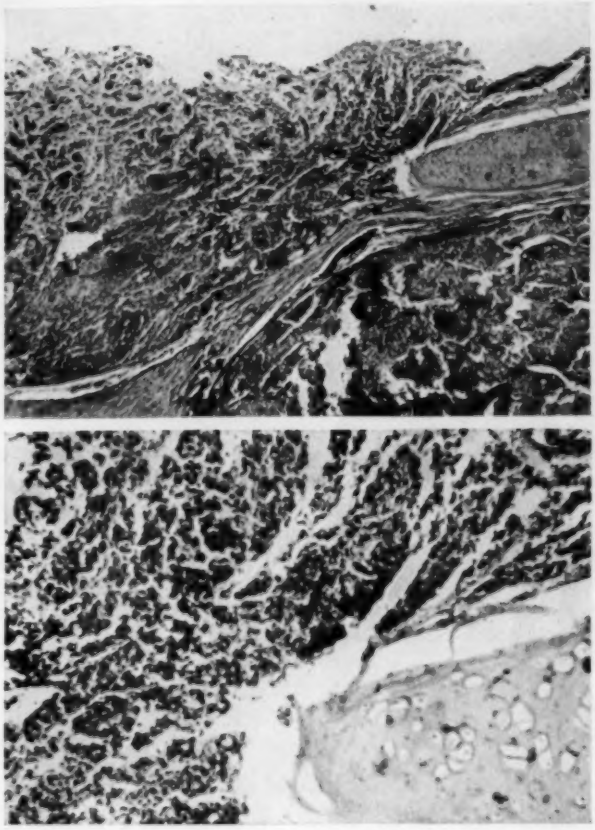

Fig. 1 (case 1).—Epidermoid carcinoma of a bronchus, metastatic from a carcinoma of the larynx. At autopsy there were a stenosing bronchial lesion, an abscess of the lung and a mediastinal metastasis. This growth was thought at first to be primary in the bronchus. Above: a longitudinal section of the bronchus; × 20. Below: a part of the same; × 100.

that there was a large mass in the *right* side of the neck, that the growth in the lung was on the *left* and that there was no definite chain of lymph nodes connecting the two. As a routine check-up, microscopic section and the record of the original tumor were examined. The biopsy speci-

men had been taken from the esophagus at about the level of the larynx and was diagnosed as epidermoid carcinoma; its cells were indistinguishable from those in the section of bronchus. Subsequently roentgen ray treatment had been given, and the mucosal lesion of the esophagus had been observed to disappear.

This was undoubtedly an instance of metastasis to the bronchus and lung from a carcinoma primary in the esophagus. The primary tumor had healed completely as a result of roentgen ray therapy, leaving tumor tissue viable only in the metastases.

Involvement of the bronchial mucosa is not a satisfactory criterion for stating that a tumor is primary in the bronchus. Metastasis to the bronchial mucosa is fairly common in a wide variety of tumors. Any tumor involving the hilar lymph nodes may extend along the lymphatics directly into the mucosa, and tumor cells may reach the mucosa as a lymphatic extension from metastatic tumors brought to the lung by the blood stream.

In the other 2 cases to be cited the diagnosis was more obvious.

CASE 2

A 47 year old Negro woman underwent a radical mastectomy at the Gallinger Municipal Hospital in June 1938 for carcinoma of the breast. In February 1939 she was readmitted because of dyspnea, loss of weight and of appetite, and swelling of the left arm and hand. Autopsy showed a bronchus 1 cm. in diameter in the lower lobe of the right lung to be almost completely occluded by tumor tissue (fig. 2). There were widespread metastases in both lungs and in the hilar lymph nodes. There were tumor deposits in the axillas, and the growth there had infiltrated a tributary of the axillary vein, whence tumor cell emboli could be carried to the lung. In the lung the tumor probably grew along the lymphatic routes and reached the hilar lymph nodes, and thence reached the bronchial mucosa; or extension could have been directly from the lymphatics of the alveoli into the lymphatic channels of the bronchial mucosa.

CASE 3

A 16 year old Negro boy was admitted to the Gallinger Municipal Hospital in April 1937 because of cough and loss of weight. There was rather marked enlargement of the cervical lymph nodes. Biopsy of one of these nodes established the diagnosis of Hodgkin's disease. On a second admission, in November 1937, mediastinal masses were demonstrated by roentgenogram. Subsequently masses developed in the abdomen, and the final admission, in May 1938, was because of enlargement of the abdomen and weakness. Roentgen ray treatment was given intermittently over the neck and abdomen between the last two admissions. Death occurred in July 1938. Autopsy disclosed, in addition to Hodgkin's disease of the liver and spleen and of the lymph nodes generally, a flat nodular growth in the right main bronchus and almost complete occlusion of the bronchus to the middle lobe of the right lung by Hodgkin's tissue (fig. 3). There was also invasion of the branches of the right pulmonary artery and vein, as well as of the lung parenchyma about the hilus. The tumor growth in the bronchi, great vessels

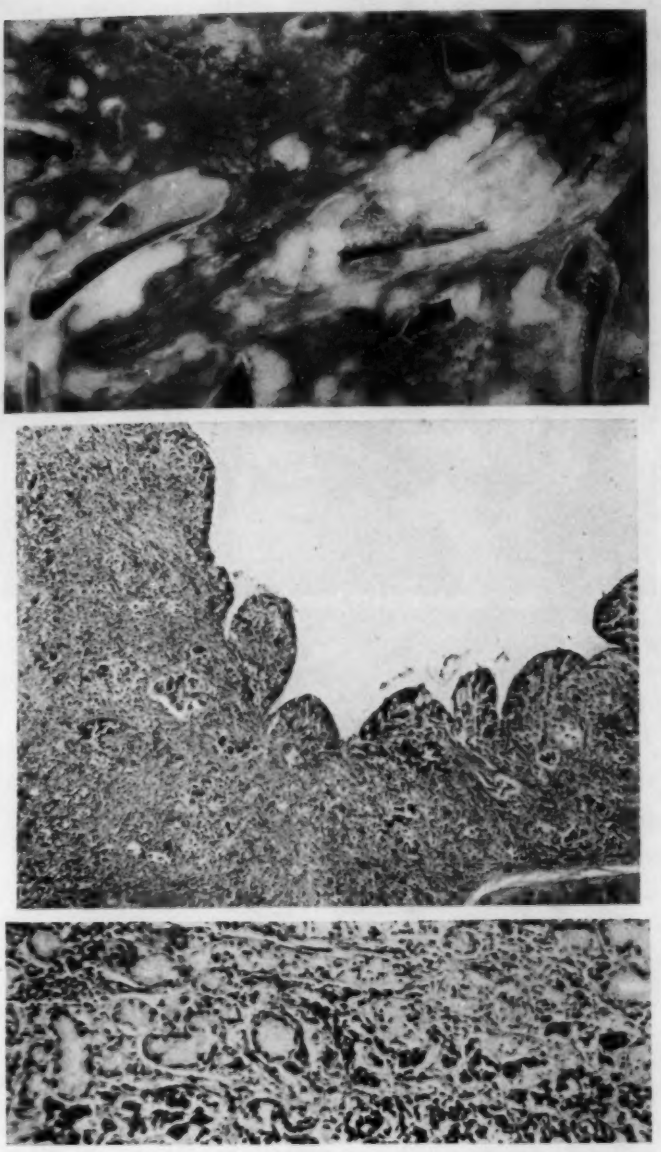

Fig. 2 (case 2).—Adenocarcinoma of a bronchus, metastatic from a tumor of a breast. Above: gross photograph, about actual size, showing flat nodular neoplastic growth filling the lumen and infiltrating the wall of the bronchus, simulating primary bronchogenic carcinoma. Middle: cross section of the bronchus, showing tumor cells in the mucosa and submucosa; × 20. Below: a part of the same, showing tumor cells infiltrating among the bronchial mucous glands; × 100.

and lung parenchyma appeared to be a result of direct extension along the lymphatics from the hilar lymph nodes.

The last 2 cases are cited not because there was any difficulty in differentiating the lesions from those of primary bronchogenic car-

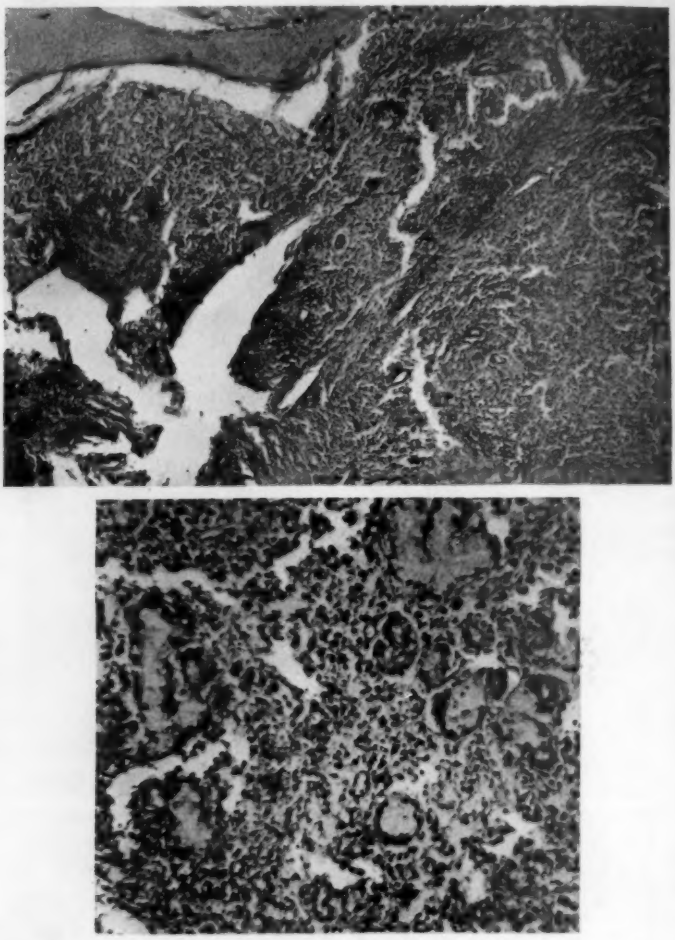

Fig. 3 (case 3).—Hodgkin's disease of a bronchus to illustrate involvement of the bronchial mucosa by growths not primary there. Above: cross section of the bronchus, the lumen of which is almost completely filled by Hodgkin's tissue; × 20. Below: a part of the same, showing Hodgkin's tissue among the mucous glands of the bronchus; × 100.

cinoma grossly but because there was extensive involvement of the bronchial mucosa by metastatic tumor. Microscopically, however, the differentiation would be more difficult. The solid sheets of rather

anaplastic cells shown in figure 2, infiltrating among the glands of the bronchial mucosa, could not be identified as from the breast by studying that section alone.

Since the relative and the actual incidence of any type of neoplasm are usually determined from autopsy statistics, the final responsibility for determining the frequency of a certain type of tumor rests on the pathologist. Generally this practice results in a high degree of accuracy, but it has its pitfalls, as most pathologists will readily admit. Autopsies do not reveal the answer to a clinical question as a cut and dried mathematical derivation, since the judgment of the pathologist and sometimes his whims and fancies come into play. Witness: the Krukenberg tumor, long classified as a primary tumor of the ovaries; primary carcinoma of the liver, which was thought to be extremely rare at one time; liposarcoma and neurosarcoma, which have emerged from the older round cell and spindle cell sarcomas thought to be of fascial origin.

It is obvious that there is no ready solution to the problem presented. The most logical approach for the pathologist is to submit every case of carcinoma of the lung encountered at autopsy to an unusually thorough investigation. If tumor masses are found outside the pulmonary system, the case should be studied very carefully to determine which is the primary lesion. It seems doubtful to me that a tumor should be considered primary in the lung when discrete nodules of equal size are distributed more or less uniformly throughout both lungs.

SUMMARY

One should hesitate in concluding that primary carcinoma of the lung is increasing in frequency. It is suggested that statistics in this field are probably misleading, for the following reasons:

1. Prior to 1900 primary carcinoma of the lung was probably diagnosed less frequently than it actually occurred, even after autopsy, because of the belief then current that the tumor was exceedingly rare. Most of the tumors of this type occurring at that time were considered metastatic, probably erroneously.

2. Possibly the diagnosis of cancer of the lung is made more frequently now than is justifiable. Involvement of the bronchial mucosa, generally considered to be one of the criteria for the diagnosis, is not reliable, since metastases to the bronchial mucosa are relatively common. The success of surgical operation and of the application of radium and the roentgen ray in eliminating accessible primary growths, while the secondary tumors remain viable and growing, places an obligation on both the clinician and the pathologist to evaluate carefully the findings in every case.

3. The virtual abandonment of the diagnoses "endothelioma of the pleura," "oat cell tumor of the mediastinum" and "tumor of the superior pulmonary sulcus" and the placing of all the tumors formerly designated by these terms in the classification of primary carcinoma of the lung, has enlarged the group considerably. These tumors appear to me to be correctly classified as primary carcinoma of the lung, but the change has swollen the statistics rather than increased the incidence of the disease.

Undoubtedly primary carcinoma of the lung is quite common, but the alarming apparent increase in the incidence of the tumor in reported statistics cannot justifiably be accepted as an actual increase until due allowances are made for the pendulum to come to rest.

EXPERIMENTAL STUDIES IN CARDIOVASCULAR PATHOLOGY

I. PATHOLOGIC CHANGES IN THE ORGANS OF RATS PRODUCED BY CHRONIC NITRITE POISONING

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An urgent need exists for additional information concerning the etiologic factors and the causative mechanism of the noninfectious degenerative and sclerosing lesions of the arterial walls and their degenerative parenchymatous sequelae in the organs involved, especially the heart, kidney and brain. Among the various endogenous factors which have been incriminated in the production of arteriosclerosis, some are of general chemical or hormonal nature (elevation of the cholesterol and calcium levels of the blood, as in diabetes and hyperparathyroidism, respectively; hyperadrenalism; renal ischemia, causing hypertension), while others are of local type (locally restricted special mechanical strain; vibrations, as at bifurcations). The experimental production of arteriosclerosis in animals by prolonged administration of cholesterol or epinephrine and by restriction of the blood flow in the renal arteries, respectively, has lent support to the contention that these factors play a causative role.

On the premise that the fundamental and general direct causative mechanism active in the development of atheromatous, arterionecrotic and arteriosclerotic lesions is represented by an impairment of the nutrition of the inner portions of the vascular walls (which are not supplied with blood by the vasa vasorum) and that this condition may be brought about by a great variety of factors acting in different ways, the postulate is advanced that hypotensive agents administered over a sufficiently long period and in adequate amounts should exert the same effect on the vascular walls as have been observed in connection with hypertensive agents. To test this hypothesis, rats were placed under the prolonged influence of a hypotension-producing chemical, erythrol tetranitrate.

This communication embraces a report on the vascular and organic changes produced by such a procedure.

Warner Institute for Therapeutic Research.

CONSIDERATIONS IN SELECTION OF EXPERIMENTAL MATERIAL

In selecting erythrol tetranitrate from the large group of nitrites and nitrates producing nitrite effects (sodium nitrite, amyl nitrite, nitroglycerin, mannitol pentanitrate, ethylene glycol dinitrate, erythrol tetranitrate and others) special consideration was given to the fact that this chemical causes not only an appreciable but a prolonged lowering of the blood pressure, in contrast to other nitrites, which have a more transitory effect in this respect. By employing this chemical, it was thought, animals could be kept for the greater part of the day under the influence of nitrite action.

Rats were chosen as test animals since they spontaneously acquire arteriosclerotic lesions in vessels of various organs (heart, lungs, kidney, testis) with old age, thus displaying a vascular reactivity similar to that observed in man, with whom, moreover, they have in common an exposure to nutritive influences of the same type, both being omnivores. The selection of rats was further influenced by the fact that recent pathologic and histologic investigations (Hueper [1]; Wilens and Sproul [2]; Hummel and Barnes [3]) have provided detailed knowledge concerning the incidence as to age, the character and the extent of the arteriosclerotic lesions occurring in rats. These animals have been used successfully, moreover, for the production of experimental hypertension by the method of renal ischemia developed by Goldblatt.[4]

EXPERIMENTAL PROCEDURE

Three experiments were conducted. In the first, 30 male rats, approximately 2 months old and weighing between 52 and 110 Gm. (average, 77 Gm.), were placed on a stock diet adequate in all respects. Increasing amounts of erythrol tetranitrate were added to the food. The chemical was obtained in tablets (tetranitrol, Merck) containing 0.015 Gm. each. As erythrol tetranitrate is sparingly soluble in water (1:20,000) but readily soluble in alcohol, a suitable number of tablets were dissolved in 95 per cent alcohol, leaving an insoluble mineral residue. This solution was shaken vigorously, after which approximately 5.0 cc. was removed and added to the food in each cage, containing 6 rats. The initial daily dose per rat was 0.00035 Gm. of erythrol tetranitrate. The dose was increased every week by 25 per cent of the amount given during the preceding week. At the end of the experimental period of thirty-two weeks the daily amount of erythrol tetranitrate administered with the food per rat had reached 0.064 Gm., an increase of approximately twenty times.

Inasmuch as the conditions prevailing in the experiment with chronic oral administration neither insured exact control of the dose consumed by the individual animal nor permitted determination of the quantity of nitrite actually resorbed into the organism, a series of 12 female rats was given subcutaneous

1. Hueper, W. C.: Arch. Path. **20**:708, 1935; **27**:466, 1939.
2. Wilens, S. L., and Sproul, E. E.: Am. J. Path. **14**:177, 1938.
3. Hummel, K. P., and Barnes, L. L.: Am. J. Path. **14**:121, 1938.
4. Goldblatt, H.: Am. J. Path. **15**:619, 1939; Bull. New York Acad. Med. **14**: 523, 1938.

injections of a saline suspension of erythrol tetranitrate over a period of one hundred and forty days (twenty weeks) on five days of the week (a total of eighty injections). The dose administered daily was 0.014 Gm. during the first week and 0.028 Gm. thereafter for the remainder of the experiment. The injections were interrupted on four occasions for the duration of one week, to permit healing of ulcerative lesions formed as the result of the subcutaneous introduction of the suspended solid material. The rats in this experiment were approximately 3 months old at the start and weighed from 118 to 158 Gm. (average, 124 Gm.).

The third experiment was devised to permit study of the organic and especially the cardiovascular effect of massive doses of an inorganic nitrite, sodium nitrite, for purposes of comparison. Six rats were used, weighing between 160 and 236 Gm. at the start of the experiment. They were placed on a meat diet, to which 0.4 Gm. of sodium nitrite per rat was added daily. After two weeks the daily dose was decreased to 0.20 Gm., as the rats did not consume their entire ration. After another week it was doubled again and three weeks later was increased to 0.5 Gm. The rats were maintained on this diet until the last survivors of the series were killed, after being in the experiment for eighteen weeks.

BIOLOGIC OBSERVATIONS

Sixteen of the 30 rats placed on chronic oral administration of erythrol tetranitrate survived for thirty-two weeks. They were then killed by bleeding from the jugular vein. During the experimental period a constant rise in weight was recorded, ranging at the time of death (when they were approximately 9 months old) from 200 to 376 Gm. (average, 262 Gm.). Nine rats of this series died or were killed during the first three months of the experiment because of an apparent inflammatory infectious condition of the middle ear, while 5 additional rats died during a later part.

Of the rats given subcutaneous injections, 10 were alive at the end of the experiment. The animals gained only slowly, their weights remaining stationary during the first two months. There was a subsequent gain which brought the weights at the end of the experiment to between 175 and 212 Gm. (average, 199 Gm.). In the course of the experiment several rats became aggressive and vicious, engaging in frequent and violent fights, especially after the injection of the nitrate.

The rats of the sodium nitrite series were listless and exhibited a cyanotic color of skin, which was especially prominent in the tails, ears and feet. All rats except one showed a more or less marked loss of weight. Two rats of this series died during the first two weeks of the experiment, and 2 additional ones were killed after being in the experiment for four weeks.

OBSERVATIONS AT AUTOPSY

Macroscopic Changes.—The rats belonging to the two erythrol tetranitrate series had relatively few macroscopically demonstrable organic lesions. In the oral series several rats which died in the course of the experiment had hyperemic meninges, small flabby brown livers, enlarged, greenish brown kidneys and small soft testes. In 7 other rats there were yellowish to greenish yellow abscesses involving the occipital region of the brain, adjacent to the region of the middle ear. The rats belonging to the sodium nitrite series showed, on the other hand, meningeal vessels filled to the point of congestion with brownish blood, flattened cerebral gyri, collapsed brownish lungs, a soft brown liver, dark red kidneys, small testes and coffee ground–colored mucosal erosions in the stomach, which contained similarly colored material. The intestine was congested and in places was filled with hemorrhagic material.

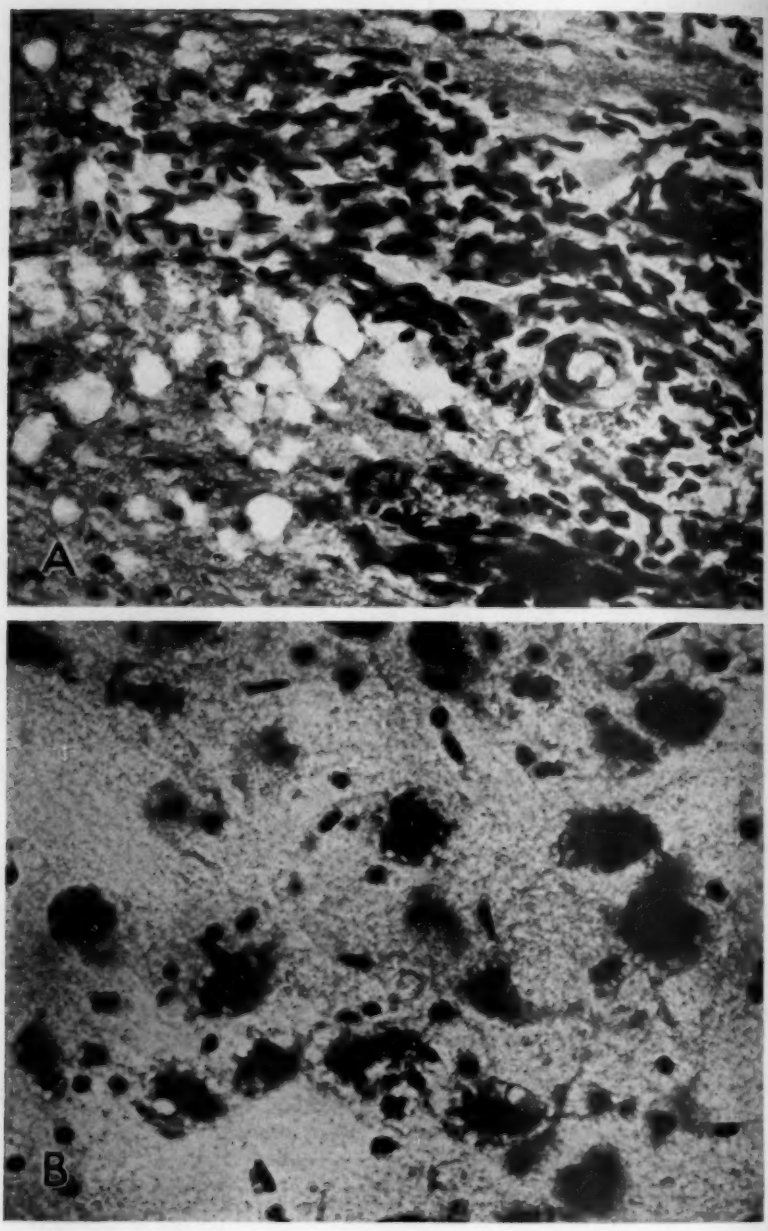

Fig. 1.—*A*, perivascular fibroblastic proliferation and lymphocytic infiltration with marked edema of the surrounding nerve tissue; × 450. *B*, extensive vacuolar degeneration of ganglion cells in a center of the brain stem; × 450.

Microscopic Changes.—Inasmuch as the histologic changes observed in the organs of rats of all three series were essentially identical, varying mainly in degree and extent, it appears appropriate to present them together, especially as they supplement each other in certain respects. Sections were prepared from the following organs: brain, heart, lung, aorta, thyroid, submaxillary gland, stomach, intestine, pancreas, liver, spleen, lymph nodes, adrenal, kidney, bladder, prostate, seminal vesicles, epididymis, testis and bone marrow of the sternum and femur. The sections were stained with hematoxylin-eosin; sections of the brain were stained in addition with toluidine blue; sections of the kidney and heart were prepared with Van Gieson's connective tissue stain; sections of the spleen and kidney were stained by the prussian blue method for iron-containing pigments.

(*a*) Brain: If the brains of 8 rats containing abscesses in the occipital region are excluded from further consideration, as these lesions could be ascribed to infections of the middle ear, the following changes were observed in the majority of animals examined: The meningeal membranes were often edematous and cellular and contained engorged vessels. Intrameningeal hemorrhages were seen occasionally. Focal or more or less diffuse lymphocytic infiltrations of the meninges were rather common. The intracerebral vessels were often congested and surrounded either by perivascular edema or small hemorrhages. Perivascular glia cell accumulations and fibroblastic proliferations were found not infrequently (fig. 1 *A*). Endothelial proliferation of the precapillary and capillary vessels was marked in some rats, causing obliteration of the lumens. In a considerable proportion of the rats the nervous substance contained either local or more or less diffuse glial cell infiltrations, involving mainly the region of the brain stem and of the cerebellum. There were small focal necroses with ameboid gliosis in several rats. Edema of the brain, sometimes associated with marked vacuolation (Swiss cheese-like picture), was observed in several animals. These lesions were often complicated by acute and chronic degenerative changes of the ganglion cells, especially those composing the centers of the brain stem (fig. 1 *B*).

(*b*) Heart: While the myocardium and coronary vessels were normal in some of the rats, many of the animals exhibited degenerative myocardial as well as coronary vascular lesions. In a few animals these changes consisted of marked vascular congestion and multiple small hemorrhagic or plasmatic extravasations. In addition to myocardial edema, there was usually an increase of interstitial histiocytic cells. Hyaline and vacuolar myocardial degenerations were found in a moderate number of rats, whereas focal fibroblastic scars existed in several (fig. 2 *A*). The lesions most frequently found in the hearts of these animals, however, involved the walls of the coronary myocardial vessels. The media was swollen and completely or partially hyalinized. The nuclei were much enlarged and irregularly round and were sometimes surrounded by a vacuolar zone. In addition to a frequent reduction in the number of nuclei, there was often a localized complete loss of these elements. Histiocytic cells were occasionally seen within the thickened, hyalinized media. The lumens of some of these pathologic vessels were narrowed or showed a compressed oblong shape (figs. 2 *A* and 3 *A*). Perivascular edema was seen in a moderate number of animals.

(*c*) Lung: An appreciable number of rats had numerous polypous as well as plaquelike calcifications in the subendothelial spaces as well as in the media of large and small branches of the pulmonary artery. The base of the calcified spurs was usually located within a break of the media. In some small arterioles the calcified projections were so large that they occluded the greater part of the lumen. The nuclei of the smooth muscle cells located in the stumps of the media were irregularly arranged in small clusters, and fibroblastic cells were interspersed with the muscle cells. The pulmonary parenchyma was normal in general.

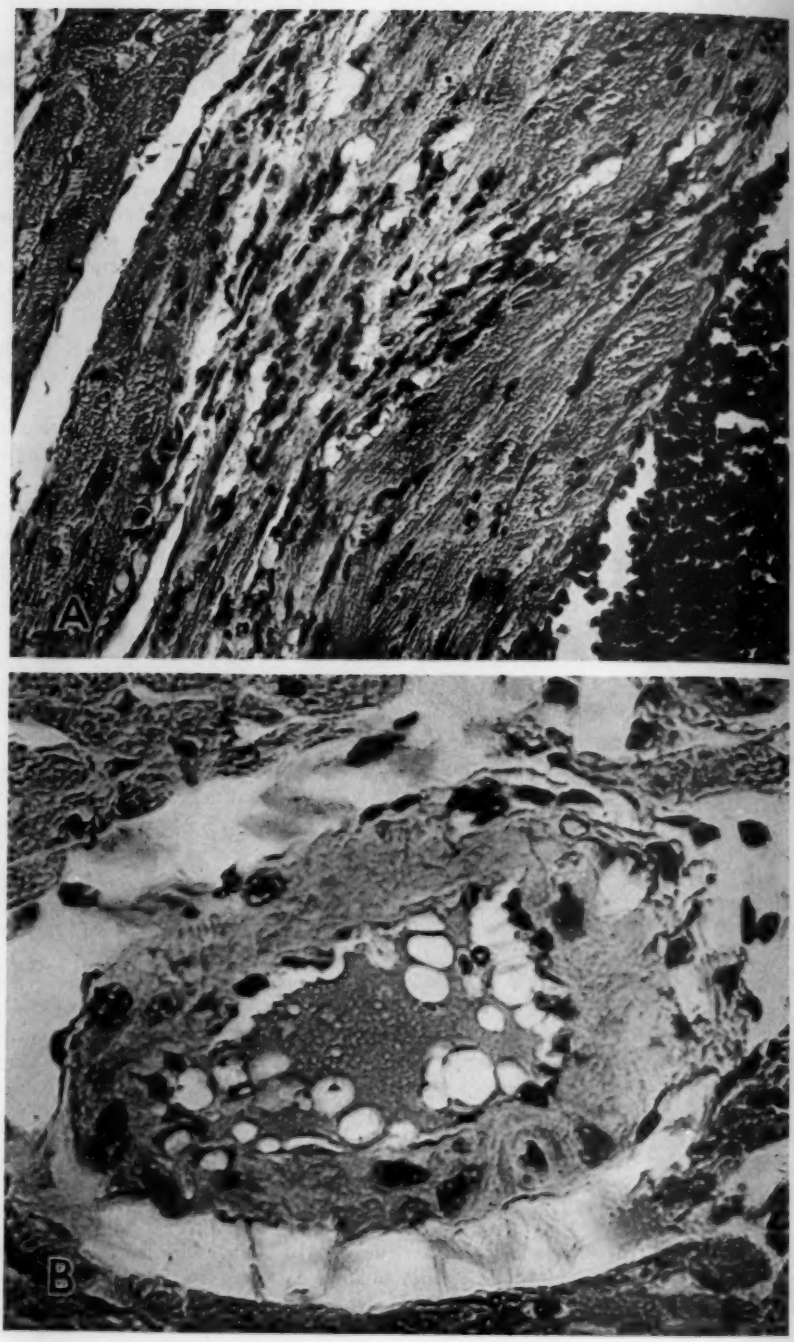

Fig. 2.—*A*, fibroblastic myocardial scar; × 345. *B*, myocardial arteriole with thickened, homogeneous wall and perivascular edema. The nuclei are reduced in number, markedly swollen and distorted. The localized crowding of nuclei is caused by an invasion of histiocytes into the degenerated and vacuolated media; × 625.

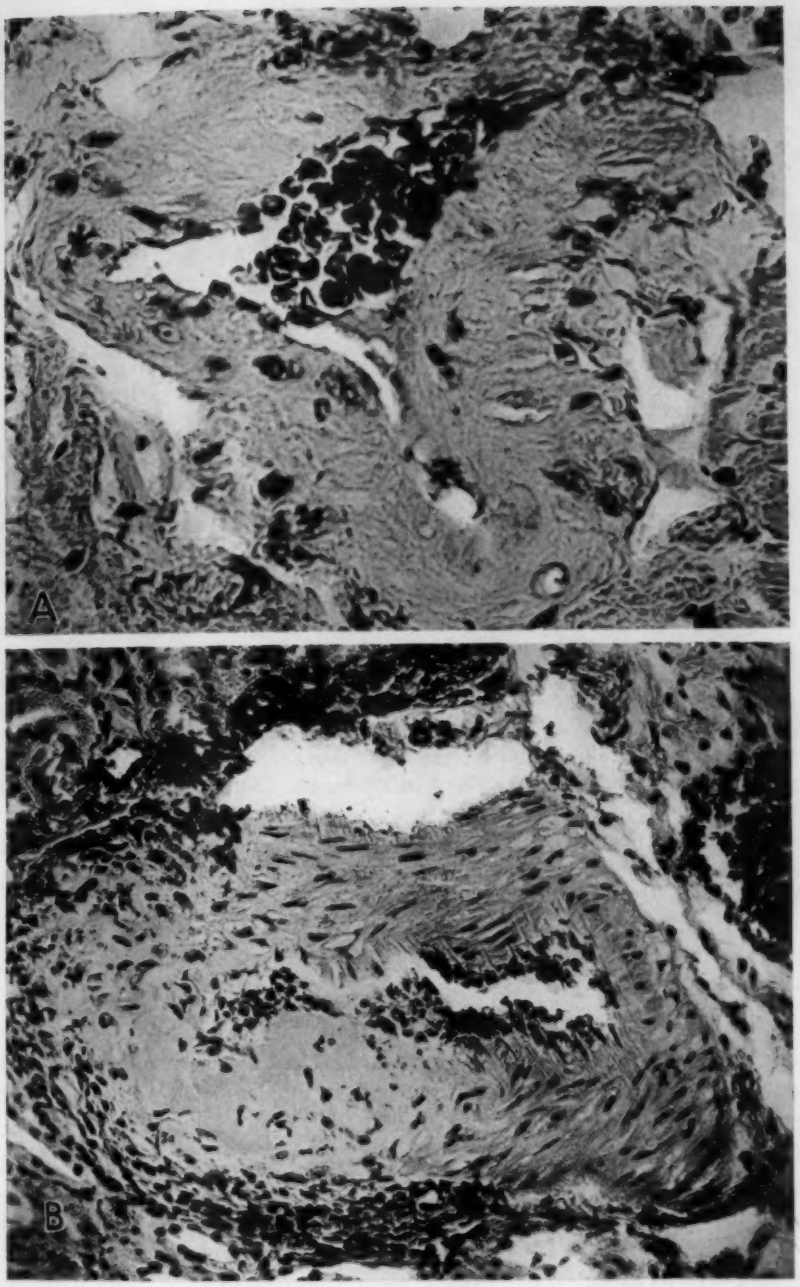

Fig. 3.—*A*, medium-sized myocardial artery with collapsed lumen and markedly thickened and hyalinized wall; × 450. *B*, large renal artery near the hilus with localized thickening and hyalinization of the wall, × 230. A few leukocytes and histiocytes are present in the marginal zone of the necrosis.

(*d*) Stomach: The gastric mucosa was normal in the rats of the two erythrol tetranitrate series, whereas it was congested and contained small hemorrhagic defects in the animals of the sodium nitrite series.

(*e*) Liver: The liver cells of most of the rats showed a peculiar fine granular or vacuolar degeneration of the cytoplasm. The nuclei often seemed to lie in empty spaces or to be located in a cytoplasm consisting of a loose accumulation of coarse granules.

(*f*) Spleen: The more or less markedly congested pulp contained in most instances a moderate to large amount of amorphous brown pigment located intracellularly and extracellularly. The brown granules stained greenish blue with the prussian blue method, while the delicate reticulum was impregnated with a dark blue iron-containing compound.

(*g*) Kidney: Frequently the tubular epithelium showed hyaline, granular degeneration and contained brownish globules which gave a negative reaction by the prussian blue method. Several of the large renal arterial branches at the hilus had a markedly thickened and locally hyalinized wall, the hyalinized area being demarcated from the intact smooth muscle cells of the media by small accumulations of mononuclear and lymphoid cells (fig. 3 *B*). The walls of the renal arterioles were not infrequently markedly thickened and contained vacuolated and swollen smooth muscle cells and subendothelial and medial hyalinizations. The lumens were narrowed (fig. 4 *A*). Several kidneys contained wedge-shaped areas in the cortex, consisting of a thickened interstitial tissue infiltrated with lymphocytes, atrophic and cystic tubules partly filled with a homogeneous, faintly calcified material and congested or atrophic and fibrotic glomeruli (fig. 4 *B*).

(*h*) Testes: In the testicular arteries of an appreciable number of rats calcified plates involved the media, occupying the entire circumference of the vessel. Some of these rats as well as some without the vascular changes showed more or less marked degenerative lesions of spermatogenic epithelium of the tubules. Spermatid giant cells and calcium incrustations in the tubular debris were seen in several instances.

(*i*) Bone Marrow: The marrow of the sternum and femur was often congested and was composed in the majority of cases of a dense myeloid tissue, in which the mature myeloid elements predominated. The erythropoiesis was usually moderate to marked. An immature marrow was seen in some instances.

(*j*) Other Organs: These were essentially normal with the exception of varying degrees of congestion.

The recorded observations demonstrate that an appreciable number of the rats treated for varying periods with erythrol tetranitrate showed degenerative lesions in the myocardial arterioles. These were associated sometimes with regressive or histiocytic-fibroblastic changes in the myocardium. Occasionally, capillary hemorrhagic extravasations were seen in a congested and edematous myocardial interstitial tissue. A considerable proportion of the rats also exhibited calcifications of the subendothelial spaces and media of the arterial vessels of the lungs and testes. Arteriolosclerotic lesions were not infrequently found in the kidneys. Regressive cellular changes were present in a large number of livers, especially among those from rats which survived for the entire experimental period. The spleens often showed evidence of chronic

passive congestion associated with destruction of erythrocytes, indicated by the large amount of iron-containing brown pigment found in many of these organs. In some of the testes extensive regressive changes

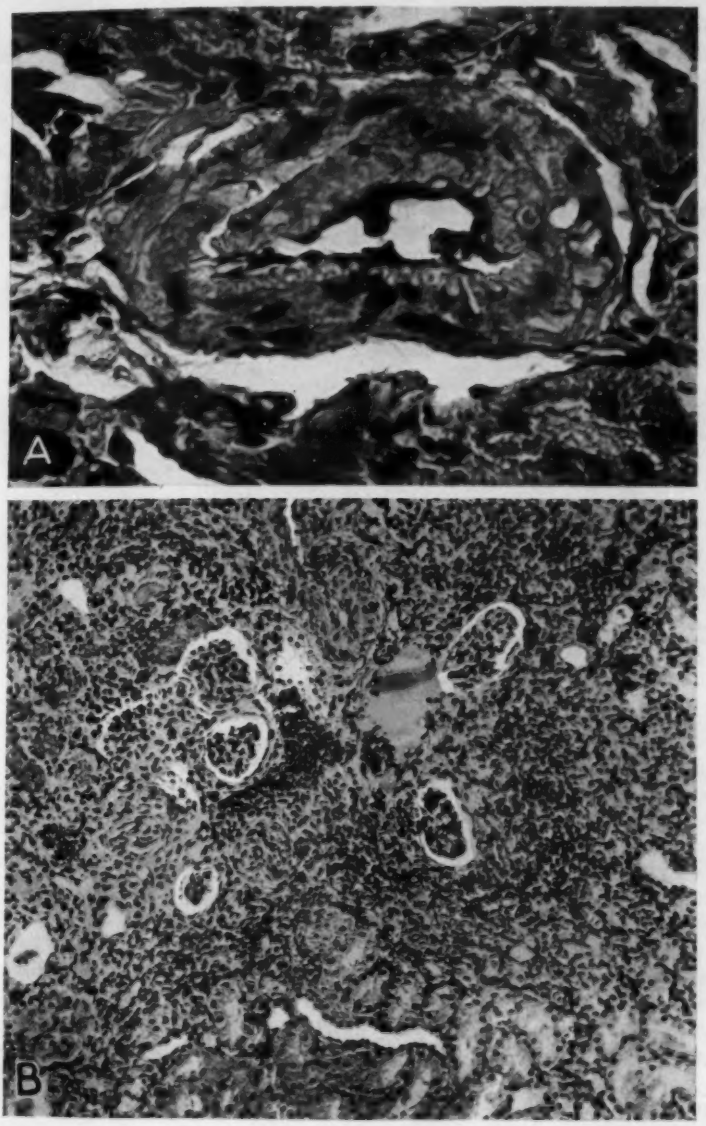

Fig. 4.—*A*, small renal arteriole with markedly thickened wall and narrowing of the lumen; × 625. *B*, area of renal scarring with atrophic, fibrotic glomeruli, lymphocytic interstitial infiltration, fibroblastic proliferation, thickening of arteriolar walls, cystic distention of tubular lumens; × 110.

of the spermatogenic epithelium were noted. The bone marrow in general displayed active erythropoiesis in the presence of an often rather mature but dense myeloid tissue. Hemorrhages and edema observed in a few cases, as well as hemosiderin in one instance, pointed to disturbances affecting both the circulation and the erythrocytes. The circulatory, vascular, glial and ganglionic changes found in the brains of an appreciable number of rats were a striking feature of the various pathologic lesions noted in these rats after the chronic introduction of erythrol tetranitrate by mouth. An analysis of these data in relation to the duration of exposure to this agent brought out the fact that the various pathologic lesions recorded increased in frequency and severity with the time of exposure.

TABLE 1.—*Summation of Averaged Findings on the Blood of Rats After Chronic Oral Administration of Erythrol Tetranitrate*

Days of Experiment	Erythrocytes, Millions	Hemoglobin, Gm. in 100 Cc.	Mean Corpuscular Hemoglobin, Gm. in 100 Cc.	Leukocytes, Thousands
1	6.74	10.4	15	18.7
15	7.41	14.9	20	16 3
35	6.64	17.5	28	18.5
61	7.15	15.3	21	15.9
77	6.95	12.8	19	19.9
106	8.57	13.5	15	24.0
119	8.83	13.3	16	31.7
127	7.85	14.2	19	28.4
152	8.43	15.3	18	30.1
190	7.76	11.9	16	25.1
213	7.75	11.1	17	26.0
222	8.31	12.8	16	16.6

HEMATOLOGIC DATA

During the course of the experiment, studies were made on the blood of some of the rats of the two series which received erythrol tetranitrate. The hematologic examinations were started with 10 rats of the oral series (only 6 survived the experimental period) and an equal number of the subcutaneous series. The examinations were made at rather irregular intervals, which in general became longer with the duration of the investigation. The blood for the examination was obtained by cutting the tails. The blood constituents studied were the leukocytes (including the differential count), erythrocytes and hemoglobin. From the figures obtained for the two last mentioned elements, the value of corpuscular hemoglobin was calculated.

The results of the hematologic studies are presented in tables 1 and 2. The tables are constructed on the basis of averaged values obtained from the 6 and 10 rats, respectively, which could be followed through the entire period of nitrite treatment. This procedure was adopted because all the animals in a particular series showed the same trend. An analysis of the hematologic data shows the following facts:

Oral Series.—The hemoglobin started to rise on the fifteenth day of the experiment and reached its peak of 17.5 Gm. on the thirty-fifth day. The values receded gradually during the rest of the experiment but always remained above the original level.

During the early period the erythrocyte counts fluctuated about a range within that given for normal rats.[5] This was followed by a marked elevation, which reached the highest peak on the one hundred and nineteenth day with 8,830,000 cells. Although the total red cell count dropped somewhat afterward, it fluctuated and maintained a mean definitely higher than the mean established for normal rats.

The mean value of the corpuscular hemoglobin showed changes similar to those in the values of hemoglobin. Following a rapid rise, which not only paralleled but surpassed the upward movement of the values of hemoglobin, there occurred a rather rapid drop in the amount of the corpuscular hemoglobin, which reached its lowest point on the one hundred and sixth day, when it was somewhat below the normal range. The latter part of the experimental period was thus characterized by the fact that after marked fluctuations in the hemoglobin and the corpuscular hemoglobin values during the first part and considerable increases of

TABLE 2.—*Summation of the Averaged Findings on the Blood After Subcutaneous Injection of Erythrol Tetranitrate*

Days of Experiment	Erythrocytes, Millions	Hemoglobin, Gm. in 100 Cc.	Mean Corpuscular Hemoglobin, Gm. in 100 Cc.	Leukocytes, Thousands
1	7.23	16.2	22	18.7
3	6.67	14.7	22	14.4
5	6.53	13.2	19	14.5
9	6.03	12.5	21	13.8
14	5.52	11.2	20	26.4
20	4.91	11.5	24	15.6
36	6.16	9.7	18	30.3
57	6.53	11.8	16	29.0
73	8.07	13.0	16	38.9
79	6.56	12.9	20	34.1
86	7.12	9.0	13	35.7
107	6.72	11.1	17	34.5
126	7.47	9.9	13	42.5
133	7.31	11.0	15	22.1

the erythrocytes during the middle part, the three constituents became more or less stabilized toward the end, remaining somewhat above the original levels in a parallel movement.

The total leukocyte count, after relatively minor fluctuations during the first two months of the experiment, showed a definite increase, which continued for the remaining five months. The peak was reached on the one hundred and nineteenth day, when a maximum of 31,730 leukocytes was recorded. From this period until almost the end of the experiment the total white cell count maintained itself at a level high above the normal range. On the day that the rats were killed, however, the white cell count had dropped to 16,640 (twelve days after the discontinuation of the nitrite treatment).

The differential count did not show any significant shifts from the normal in spite of the considerably increased number of leukocytes present during the latter half of the experimental period.

It was observed that in most instances the number of reticulocytes was increased definitely after the administration of erythrol tetranitrate, while the number of platelets exhibited a tendency toward lower values, within the normal range, during the latter part of the experimental period.

5. Wintrobe, cited by Cushny.[6]

Subcutaneous Series.—The values of hemoglobin dropped almost steadily from the start of the experiment, reaching their lowest figure (9.76 Gm.) at the end of the fifth week. Following a transitory rise, the values dropped again and reached their lowest figure (9 Gm.) at the end of the third month. There was subsequently a moderate rise with values somewhat below the normal range (i. e., between 9.9 and 12.9 Gm.).

The erythrocyte count exhibited a similar initial reduction, which was also followed by a gradual rise. This, however, was more prolonged than that of the values of hemoglobin. After having reached a figure slightly above the original one, the erythrocyte count decreased again and remained for the rest of the experimental period within normal limits.

Following a slight initial drop and a subsequent steep rise to somewhat above the normal level, the corpuscular hemoglobin decreased severely and persistently and remained at a reduced level for the last part of the experiment.

The leukocytes remained stationary as to number and type during the first quarter of the experimental period, then rose constantly until they reached their peak before the end of the experiment, when 42,470 white cells represented the average leukocyte count. There was at this time a moderate increase in the proportion of the neutrophilic and especially of the juvenile leukocytes.

COMMENT

The significance of the recorded vascular lesions and of the parenchymatous organic alterations depends on the demonstration of causative interrelations between the morphologic phenomena and the administration of erythrol tetranitrate and sodium nitrite, respectively. Inasmuch as Hueper,[1] Wilens and Sproul [2] and Hummel and Barnes [3] reported the spontaneous occurrence of similar cardiovascular lesions in rats, it is essential that satisfactory evidence be supplied which will exclude a possibly senile character of the organic changes observed in the rats treated with nitrites. The observations made by Wilens and Sproul [2] on the age distribution of these pathologic changes of the circulatory tissue of rats are therefore of great importance. These investigators found that rats less than 400 days old are practically free from degenerative cardial lesions, while the coronary sclerotic changes noted in 23 of 487 rats examined showed in their age incidence a direct relation to the senile period. Similar observations were made by Hummel and Barnes, who noted that sclerotic cardiovascular lesions were found in only 2 of 111 rats dying at an age of 261 to 374 days. As the animals comprising the nitrite series were in no instance older than 300 days, i. e., they belonged to an age period during which normally degenerative cardiovascular changes are rare or absent, it becomes obvious that the organic lesions observed cannot be considered as the physiologic accompaniments of old age but must be regarded as the results of the nitrite treatment. This conception is supported by the fact that they occurred in the rats not only with marked severity and high incidence but with increasing frequency corresponding to the duration of exposure to the nitrites.

A possible senile nature of the lesions observed in the treated rats having been excluded, it becomes pertinent to demonstrate whether the symptomatic and morphologic manifestations noted in the experimental animals resulted from the pharmacologic and toxicologic effects known to be exerted by nitrites and whether these reactions were similar to or identical with those seen in chronic nitrite poisoning of man.

The functional effects which inorganic nitrites as well as inorganic and alkyl nitrates exert on the vascular walls and the circulation of the blood are expressed in a relaxation of the walls of the peripheral arterioles with a resultant drop in blood pressure. The most susceptible vessels, which react first and to the smallest doses, are those of the head, neck, brain and meninges. Larger doses cause relaxation of the coronary vessels, while still larger ones produce dilatation of the vessels of the splanchnic region. It is this widening of the vascular bed which causes the drop in blood pressure. The slowing of the blood flow results in congestion, which in turn is followed by development of hypoxemic states, especially in those organs in which the demand for oxygen is high or which are highly susceptible to even minor deficiencies in the oxygen supply (brain and heart). It has been noted that the degree of vasodepression elicited depends not only on the particular type of nitrite used and the dose given but to a great extent on the subject as well as on his age (Cushny [6]; Löwy [7]; Prodger and Ayman [8]; Sprague and White [9]).

The ensuing subjective symptoms of vasculocirculatory disturbances observed in chronic nitrite poisoning are: transitory or more or less persistent low blood pressure, which may drop below 80 mm. of mercury; a weak, soft pulse; dilatation of the left side of the heart; accentuation of the first heart sound; exaggeration of the apical beat; murmurs similar to those found with anemia, aortic regurgitation or mitral insufficiency; irregularity of the heart beat, and other signs of myocardial impairment. Tachycardia or bradycardia is often present, the latter condition, however, changing readily into tachycardia on slight physical exertion, excessive change in environmental temperature and humidity or consumption of alcoholic beverages or other agents which exert an additional vasodepressant effect. Precordial pain and anginoid attacks are not infrequently recorded. During more acute episodes the color

6. Cushny, A. R.: Die Nitritgruppe, in Heffter, A.: Handbuch der experimentellen Pharmakologie, Berlin, Julius Springer, 1923, vol. 1, p. 833; A Text Book of Pharmacology and Therapeutics, ed. 11, Philadelphia, Lea & Febiger, 1936, p. 630.

7. Löwy, J.: Die Klinik der Berufskrankheiten, Vienna, Emil Haim & Co., 1924, p. 392.

8. Prodger, S. H., and Ayman, D.: Am. J. M. Sc. **184**:480, 1932.

9. Sprague, H. B., and White, P. B.: M. Clin. North America **16**:895, 1933.

of the skin is purplish livid, most noticeable in the nail beds, lips, ears and nose. As the result of the direct or indirect action of the nitrites on the cerebral circulation there occur, in addition to headache, diverse symptoms such as insomnia, restlessness, maniacal episodes, states of drowsiness and mental dulness. The vasodepressant effect on the splanchnic system causes the appearance of symptoms of indigestion and gastroenteritis (colic, bloody diarrhea) resulting from chronic congestion and hemorrhages of the gastric and intestinal mucosa. Sudden death may ensue with the symptoms of cerebral paresis (Meixner and Mayrhofer [10]) or of coronary thrombosis (International Labor Office Report [11]; Löwy [7]; Laws [12]; Ebright [13]; Robert [14]; Schulz,[15] and others).

The following macroscopic and microscopic lesions have been recorded in connection with deaths from nitrite poisoning: meningeal and cerebral edema and congestion with perivascular edema and accumulation of lymphoid and glia cells and brown pigment; multiple cerebral hemorrhages affecting mainly the region of the brain stem; intimal proliferation, medial hyalinization, swelling and calcification of the cerebral vessels; perivascular fibrous scars with capillary proliferation, gliosis and blanching of the nerve substance; glial scars; acute and chronic degeneration of ganglion cells, involving chiefly the centers of the brain stem; coronary sclerosis; swelling and hyalinization of the walls of the myocardial arterioles; enlargement of the heart with hypertrophy of the left ventricular wall; myocardial degeneration, fibrosis and cicatrization; chronic passive congestion of the liver with pericentral fatty degeneration; hemorrhagic gastritis and enteritis; nephrosis; arteriosclerosis of the renal vessels, and pancreatic hemorrhages (Solis-Cohen and Githens [16]; Löwy [7]; von Jaksch [17]; Binz [18]; Meixner and Mayrhofer [10]; Fischer-Wasels,[19] and others).

During the course of the experiment several of the listed symptomatic manifestations of chronic nitrite poisoning, such as cold livid skin, mental depression, aggressiveness, as well as fainting spells, and attacks of tachycardia, were observed following the injection of nitrite. The

10. Meixner, K., and Mayrhofer, A.: Vrtljschr. f. gerichtl. Med. **61**:228, 1921.
11. International Labor Office Reports, vol. 2, no. 281, Geneva, Switzerland, International Labour Office.
12. Laws, G. C.: J. A. M. A. **31**:793, 1898.
13. Ebright, G. E.: J. A. M. A. **62**:201, 1914.
14. Robert, E.: J. de méd. de Bordeaux **112**:10, 1935.
15. Schulz, O.: Samml. f. Vergiftungsfälle (B) **6**:59, 1935.
16. Solis-Cohen, S., and Githens, T. S.: Pharmacotherapeutics, New York, D. Appleton and Company, 1928, p. 370.
17. von Jaksch, cited by Löwy.[7]
18. Binz, C.: Arch. f. exper. Path. u. Pharmakol. **13**:133, 1881.
19. Fischer-Wasels, B.: Frankfurt. Ztschr. f. Path. **45**:1, 1933.

hematologic findings reflected the changes produced in the circulation and oxygenation of the blood. The hematologic effects were especially striking in the oral group. Following a primary compensatory phase of hemoglobinosis, there developed an appreciable and relatively continuous erythrocytosis in response to the persistent and possibly intensified circulatory hypoxemia produced by the administration of nitrites. Confirmatory evidence of the presence of marked circulatory embarrassment during the latter part of the experimental period in both series is furnished by the behavior of the leukocytes. The considerable leukocytosis without the usually occurring shift toward a higher proportion of immature cell forms and with the presence of a predominantly mature or resting myeloid marrow characterizes the leukocytotic condition as a distributory phenomenon representing leukocytostasis in the distal, peripheral vessels, such as those of the tip of the tail, caused by excessive slowing of the blood flow.

The anatomic studies of the organs of the rats treated with nitrites revealed that the vascular and parenchymatous lesions produced in these young animals were identical with those observed in human subjects who had been exposed and subsequently succumbed to a chronic exposure to chemically related nitrites and similarly acting nitrates. It is obvious that the existence of causal relations between the regressive and sclerotic vascular alterations and some of the related parenchymatous degenerations (especially those in the brain, heart, kidney and testis) and the hypotensive and hypoxemic effect exerted by the nitrite has not been recognized in the past because the anatomic manifestations are very similar to those considered representative of, and resulting from, or accompanying, chronic hypertension. In the absence of adequate etiologic and symptomatic data these pathologic changes may have been misinterpreted or may have been considered as coincidental.

It is believed that the experimental data presented offer satisfactory evidence in support of the actual existence of such interrelations and thus furnish experimental proof of the original premise that arteriosclerotic lesions may develop on a hypotensive as well as on a hypertensive basis, provided the hypotensive condition is sufficiently severe and prolonged. While an inordinate hypertensive vasoconstriction interferes with proper nutrition of the vascular wall, especially of its inner portion, not supplied with vasa vasorum, by a reduction of the quantity of blood which can penetrate these more or less compressed vessels (ischemic hypoxemia), the same effect is produced by the stagnant hypoxemia resulting from the excessive slowing of the blood flow accompanying hypotensive vasodilation, which also affects mainly those parts of the vascular walls in which vital activity depends on nutritive exchange and discharge of waste products through the process of diffusion.

SUMMARY

Oral and subcutaneous administration of erythrol tetranitrate and sodium nitrite, respectively, to young, immature rats over a period of several months results in the production of degenerative vascular and parenchymatous lesions in the heart, lung, brain, kidney and testis.

The chief causative factor in the development of these changes is represented by a stagnant hypoxemia resulting from the slowing of the blood flow caused by the hypotensive vasodilatation elicited by the nitrite.

These experimentally produced organic changes are identical with those seen in man following chronic nitrite poisoning and are very similar to those observed in spontaneous or experimental hypertension as found in man and animals, respectively.

Inasmuch as vasodilating agents as well as vasoconstricting agents cause a nutritive disturbance of the vascular walls, prolonged vasodilation or vasoconstriction (hypotension or hypertension) results in the appearance of degenerative and reactive proliferative phenomena in the vascular walls, which are considered as characteristic of arteriosclerosis and arteriolosclerosis, angionecrosis and atheromatosis.

The same causative mechanisms produce, by hypoxemia and associated nutritive disturbances, regressive changes in the parenchymatous organs (heart, brain, kidney, testis), which are accentuated by the progressive circulatory impairment resulting from the vascular changes.

INCIDENCE OF PRIMARY CARCINOMA OF THE LUNG

A REVIEW OF YALE AUTOPSY PROTOCOLS, 1917 TO 1937

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In an earlier report [1] on the incidence of primary carcinoma of the lung it was concluded that the generally recognized increase in the frequency of this neoplasm was real and absolute. Excellent general reviews, particularly by Fried [2] and by Simons,[3] have since been published, but there still exists marked divergence of opinion as to the nature of the observed increase in frequency of bronchiogenic carcinoma. Is this increase apparent or real, relative or absolute? After analyzing the various factors involved, Fried [2] concluded that "the increase then is very likely more apparent than real," and Frissell and Knox [4] concurred in this belief, stating that ". . . it would seem probable that . . . the increase in the incidence of bronchial carcinoma in the last two decades is apparent rather than real." On the other hand, Hill,[5] after a survey of the relevant factors, declared that "a real increase in the incidence has occurred," and more recently Matz [6] indicated his opinion that "the increased incidence of bronchiogenic carcinoma is absolute."

The problem at first glance may appear to be only of academic importance. Its resolution, however, has broad implications. If, as some believe, the increase in the number of pulmonary cancers is merely an expression of the aging of a population subjected to improved diagnostic facilities and sharper clinical acumen, little can be done by way of prophylaxis. On the other hand, if the increase is greater than can be expected on the basis of increasing numbers of persons in the older age groups, greater even than can be ascribed to more accurate diagnostic methods, attention can be directed to the elucidation and perhaps ultimate elimination of the responsible factor or factors. It is the purpose

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1. Rosahn, P. D.: Am. J. M. Sc. **179**:803, 1930.
2. Fried, B. M.: Medicine **10**:373, 1931.
3. Simons, E. J.: Primary Carcinoma of the Lung, Chicago, The Year Book Publishers, Inc., 1937.
4. Frissell, L. F., and Knox, L. C.: Am. J. Cancer **30**:219, 1937.
5. Hill, R. M.: Edinburgh M. J. **41**:320, 1934.
6. Matz, P. B.: J. A. M. A. **111**:2086, 1938.

of this paper to present new data on the subject together with a critical analysis of the reasons generally advanced for accepting the view that the increase in the incidence of carcinoma of the lung is only apparent. Pertinent data of correlative interest are also presented.

MATERIAL AND METHODS

The first autopsy in the current series at the Yale University School of Medicine was performed on Sept. 22, 1917. From this date to Dec. 31, 1937 a total of 4,156 persons were examined post mortem. The cranial cavity only was examined in 42 of these, and, of the remaining 4,114, 1,887 were males over 20 years of age and 1,081 were females over 20. The latter two groups, totaling 2,968 persons, form the basis of the present report. In the discussion, the period from Sept. 22, 1917 to Dec. 31, 1927 has been termed the first decade, and the period from Jan. 1, 1928 to Dec. 31, 1937 has been called the second decade.

The age and sex of every person with carcinoma coming to autopsy, together with the primary site, were recorded. In the vast majority of these persons the neoplasm was the primary cause of death, but in a small number carcinoma was an incidental finding at autopsy. Statistical constants were then determined for each organ or system involved, and the annual and decennial incidence calculated. For comparative purposes the age and the sex distribution of all persons in the entire autopsy series aged 20 years or older were also tabulated and statistical constants calculated. For this purpose the punched card system described elsewhere [7] proved of inestimable assistance.

The statistical methods employed were those which are in common use, and the texts of Fisher [8] and Snedecor [9] were freely consulted. When the probability of an event occurring by chance alone was 5 or less than 5 in 100 trials ($P \leq 0.05$) the occurrence of the event was said to be *significant*. If the event could occur by chance once or less than 1 in 100 trials ($P \leq 0.01$), the event was said to be *highly significant*. This is in accordance with Snedecor's terminology.

A new concept in time trend regression equations is here introduced. The accepted method of analyzing a trend in time involves the use of equal intervals of time as the independent variable. As applied to the question under discussion, the incidence of primary carcinoma of the lung, the orthodox procedure would be to plot the proportion of primary pulmonary cancers to all cancers occurring in succeeding equal time intervals. An error is thus introduced, its magnitude depending on the size of the sample. For it is self evident that 2 cases of tumor of the lung among 20 instances of carcinoma are of less significance than 6 cases among 60 instances of carcinoma, although the resulting percentages are the same in each instance. When the total number of tumors per successive unit of time varies, the error of the ratio between one type of tumor and all types fluctuates correspondingly. Moreover, successive determinations in the problem at hand need not necessarily be made on the basis of equal time intervals. What interests the observer in this connection is whether one type of tumor is changing in frequency over a given period of time. The broad vista of this period concerns him, and not the equality of the successive intervals composing it.

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7. Rosahn, P. D.: J. Tech. Methods **18**:32, 1938.

8. Fisher, R. A.: Statistical Methods for Research Workers, ed. 5, London, Oliver & Boyd, 1934.

9. Snedecor, G. W.: Calculation and Interpretation of Analysis of Variance and Covariance, Ames, Iowa, Collegiate Press, Inc., 1934.

In considering this problem it appeared reasonable to relate the incidence of carcinoma of any organ to successive equal numbers of all cancers without regard to the time period during which these equal numbers of cancers were observed. This procedure would thus eliminate the variable error resulting from the occurrence of different numbers of tumors in successive equal intervals of time. Following this reasoning, all carcinomas were numbered in sequence as they were found in the autopsy protocols, and the regional distribution of the carcinomas comprising the first, second, third and fourth hundred carcinomas was then determined. Thus the error of a proportion was eliminated, and yet the use of one hundred as a measuring rod proved to be of great convenience, since it permitted a direct translation from the absolute number of carcinomas of any one region per hundred of all carcinomas into the corresponding relative percentage.

RESULTS

A total of 435 carcinomas occurring in 425 different persons was observed in the series of 4,114 autopsies. The regional distribution of

TABLE 1.—*Regional Distribution of Carcinomas Observed in Yale University Autopsy Series, 1917-1937*

Organ Involved	1917-1937		1917-1927		1928-1937	
	Number	Percentage	Number	Percentage	Number	Percentage
Large intestine	71	16.3	21	15.4	50	16.7
Stomach	58	13.3	27	19.9	31	10.4
Lung	43	9.9	10	7.4	33	11.0
Prostate	32	7.4	5	3.7	27	9.0
Uterus	29	6.7	13	9.6	16	5.4
Esophagus	27	6.2	8	5.9	19	6.4
Breast	26	6.0	8	5.9	18	6.0
Liver and bile ducts	26	6.0	12	8.8	14	4.7
All others	123	28.3	32	23.5	91	30.4
Total	435	100.1	136	100.1	299	100.0

these neoplasms for each ten years and for the entire twenty year period is shown in table 1. During the interval from 1917 to 1927 there were 10 primary pulmonary tumors, or 7.35 per cent of the 136 carcinomas encountered. This compares with 33 primary pulmonary cancers seen in the period from 1928 to 1937, or 11.04 per cent of the 299 cancers observed. It is of interest to note that the relation of pulmonary cancers to all cancers during the period 1917 to 1927 in this report is similar to the findings [1] at the Boston City Hospital during the nearly comparable period from 1920 to 1928: 7.35 per cent in the first instance and 7.23 per cent in the second. Both of these values correspond closely to the 6.98 per cent incidence of primary carcinoma of the lung among all carcinomas found in a review [1] of the world literature for the period from 1920 to 1928.

The table indicates that during the period from 1917 to 1927 carcinoma of the lung was the fifth most frequent tumor, being exceeded in frequency by carcinoma of the stomach, large bowel, uterus and liver and bile ducts, in this order. In the following decade carcinoma of the stomach fell to third position, and carcinoma of the lung rose to second

place, being exceeded in frequency only by malignant tumors of the large bowel. It will be seen that with the exception of prostatic carcinoma, carcinoma of the lung was the only neoplasm the incidence of which increased appreciably during the second of the two ten year periods under consideration. As will be shown in a subsequent table, the increase in the incidence of prostatic carcinoma was variable, and the trend line for this tumor showed no significant deviation from zero.

Table 2 gives the regional distribution of the neoplasms comprising the first, second, third and fourth hundred carcinomas. The first hundred carcinomas were observed during the ten year period from October 1917 to September 1926, the second hundred in the three and a half year period ending March 1930, the third hundred during the nearly four year period ending January 1934 and the fourth hundred

TABLE 2.—*Regional Distribution of First to Fourth Hundred Carcinomas*

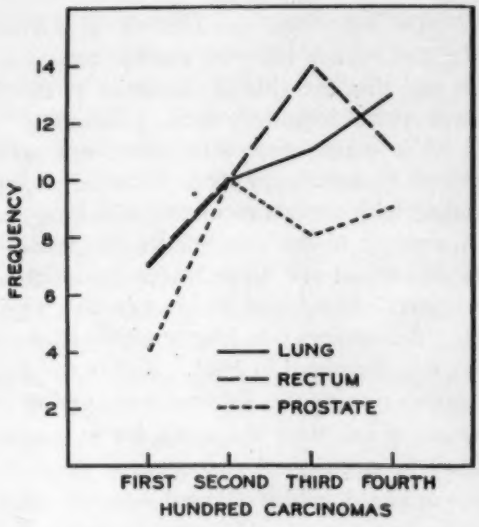
		Site								
Carcinomas	Period of Observation	Stomach	Rectum and Sigmoid	Lung	Prostate	Uterus	Liver and Bile Ducts	Esophagus	All Others	Total
1-100	October 1917-September 1926	21	7	7	4	9	6	7	39	100
101-200	September 1926-March 1930	12	10	10	10	6	9	6	37	100
201-300	March 1930-January 1934	14	14	11	8	4	7	1	41	100
301-400	January 1934-December 1936	9	11	13	9	9	4	8	37	100
1-400	October 1917-December 1936	56	42	41	31	28	26	22	154	400

during the following two year interval ending December 1936. Thirty-five additional carcinomas noted between Dec. 15, 1936 and Dec. 31, 1937 are not included in this table because they did not fit conveniently into the "one hundred carcinoma" grouping followed. The variability of the time intervals during which successive hundred carcinomas were seen is largely a reflection of the increasing annual number of autopsies performed. Only 7 persons with primary carcinoma of the lung were encountered in the period from 1917 to 1926, during which the first hundred carcinomas were encountered at autopsies. The number progressively increased to 13 in the interval from 1934 to 1936, when the fourth hundred carcinomas were observed. In the last period primary carcinoma of the lung was the most frequent of all tumors. The increasing incidence is shown graphically in the chart, which for comparative purposes shows the trends of carcinoma of the prostate and rectum during comparable periods.

The trend line for the frequency of carcinoma of the lung in each of the four successive hundred carcinomas is represented by the regres-

sion equation $Y' = 5.5 + 1.9X$, with S_{π} being equal to 0.6, and t, to 5.51. The coefficient of correlation (r) between the number of primary pulmonary carcinomas and the succeeding hundred carcinomas was + 0.9744. The foregoing values of t and of r are both within the range of significance. Such a trend as that described would be expected to occur by chance alone less than four times in a hundred similar series.

The significance of the increasing incidence of primary cancer of the lung is further enhanced by the demonstration that it alone of all tumors had a significant positive trend. Regression equations prepared in the same manner for the incidence of carcinoma of the stomach, liver and



Frequency of carcinoma of the lung, rectum and prostate, respectively, in the first to fourth hundred carcinomas in the Yale autopsy series.

bile ducts, prostate, intestine, female genitalia, esophagus and rectum, respectively, all showed no significant deviation from the horizontal.

ANALYSIS OF CERTAIN FACTORS OFTEN CITED AS EVIDENCE THAT CANCER OF THE LUNG HAS SHOWN ONLY A RELATIVE INCREASE

The arguments in favor of the belief that the generally reported increase in the incidence of primary carcinoma of the lung is relative and not absolute have been summarized best by Fried:

> The author is of the opinion that the more frequent occurrence of this disease in recent years, as compared to the older findings, can be explained on the basis of the following factors: (1) Improved clinical and pathological methods of

diagnosis; (2) Increased attention to this malady (as Goethe has expressed it, "Man sieht nur was man weiss"); (3) Increase in span of human life (a much greater proportion of people reach the "cancer age").

Frisell and Knox have added a fourth reason, also suggested by Fried:

Since external and readily diagnosed cancer has not increased, it would seem probable that, in spite of the evidence of the post-mortem figures, especially in Germany, the increase in the incidence of bronchial carcinoma in the last two decades is apparent rather than real.

Each of these factors will be discussed, the material here presented being used as a background.

1. *Improved Clinical and Pathologic Methods of Diagnosis.*—There is no doubt that the past decade has seen marked technical advances in diagnostic facilities and that the clinical diagnosis of carcinoma of the lung has been made more frequently than previously. The use of iodized poppyseed oil in conjunction with roentgenographic work, the greater knowledge of bronchoscopy and wider employment of the bronchoscope together with the thoracoscope and lung puncture have all added to the frequency of the antemortem diagnosis of carcinoma of the lung. The records of the New Haven Hospital have recently been reviewed by Kober,[10] who found 26 cases in which primary cancer of the lung had been demonstrated by biopsy without autopsy. The first tumor in this series was diagnosed in 1921. Half of the 26 tumors were diagnosed ante mortem during the fourteen year period from 1921 to 1935, while the remaining half were diagnosed during the two years 1936 and 1937. It should be emphasized that none of these 26 tumors are included in the present report, which is based solely on autopsy material. The increase in the number of pulmonary cancers diagnosed ante mortem enhances the value of the statistically significant increase in the postmortem incidence. Moreover, this postmortem increase, credited by some to be predominantly German, has also been widely recognized in this country.

Changes have also occurred in the histologic classification of primary pulmonary tumors. As Frissell and Knox[4] stated:

First, many tumors classified as sarcomata by pathologists of the last century are now included as epithelial tumors of the so-called oat-cell variety. Secondly, carcinoma of the lung, when found, was usually considered metastatic; . . . Third, the widespread interest of pathologists in this subject, particularly in the last decade, has led to the discovery of a considerable number of small pulmonary neoplasms with large metastases; such metastases earlier observers undoubtedly regarded as the primary lesion.

10. Kober, W. M.: Primary Carcinoma of the Lung, Thesis, Yale University School of Medicine, 1938.

My co-workers and I have reviewed our cases with these three points in mind. In only 2 instances was the original anatomic diagnosis altered after restudy of the slides. The autopsy in the first of these cases (A 1443) was done in 1927, and the tumor was originally called a "malignant mediastinal neoplasm." This on reexamination was found to be a typical bronchiogenic carcinoma of the oat cell variety. In the second instance (A 1705) the autopsy was made in 1928, and at that time the tumor was classified as a "squamous cell carcinoma involving pericardium, pleura and thoracic wall." On restudy this was included as a squamous cell carcinoma of the lung. Both of these tumors were in the second hundred carcinomas. Had the original diagnoses been accepted and had the tumors as a result been excluded from the series, the resulting regression equation would have had greater significance, t in this case being equal to 6.05 ($P = 0.03$—). Even though these 2 tumors were included, however, the calculated regression equation retained a significant positive deviation from zero. In this connection it should be stated that all mediastinal tumors occurring in the period under discussion were reviewed, and the aforementioned one (A 1443) was the only tumor reclassified as a primary carcinoma of the lung. In none of the other cases mentioned, with the exception of A 1705, was there any doubt that the lung was the primary site of the neoplasm.

2. *Increased Attention to This Malady.*—This statement appeared in 1931 to explain the increasing incidence of primary pulmonary tumors noted up to that time. There can be little doubt that a certain number of tumors diagnosed carcinoma of the lung in the past twenty years would previously have been assigned to some other diagnostic category not only by the clinician but by the pathologist also. But to explain the statistically significant increase in the number of pulmonary cancers in our autopsy material of the past twenty years on the basis of increased attention to this malady presupposes a constantly increasing awareness of this condition on the part of both clinician and pathologist from 1917 to 1931 and thereafter to the present. The curve of attention to carcinoma of the lung would thus have to show a positive linear trend paralleling the increasing incidence of pulmonary tumors. It would be necessary to assume also that in the years under discussion the attention to and interest in tumors of other organs were maintained at a constant level. Neither of these hypotheses is susceptible of proof, and they do not appear plausible. Since the number of publications on a subject is an excellent indication of the general interest in that subject, the *Index Medicus* for the past twenty years was examined. The number of published reports on various aspects of carcinoma has increased tremendously during this period, but what is of more importance in the present connection is the fact that carcinoma of practically every organ,

and not of the lung alone, is represented by an increased number of publications.

In this connection, it has been said that because patients with carcinoma of the lung present peculiar diagnostic difficulties, intensive efforts are expended to secure consent for necropsy in such cases. As a result, it is claimed, a greater proportion of persons with tumor of the lung come to autopsy than would otherwise be the case, accounting in part for the recorded increase in the incidence of this neoplasm. However, the difficulties involved in the diagnosis of carcinoma of the lung are probably no greater than those accompanying the diagnosis of a suspected neoplasm of any other visceral organ. The symptom complex of carcinoma of the lung is perhaps no more bizarre and no more challenging from a diagnostic point of view than a malignant tumor of the liver and bile ducts. Nevertheless, tumors of the latter system in the reported series did not increase in number during the same period when

TABLE 3.—*Mean Age of Persons Over Twenty Years of Age in Yale University Autopsy Series, 1917-1937*

	Total Series		Women *		Men †	
Period	Number	Mean Age, Yr.	Number	Mean Age, Yr.	Number	Mean Age, Yr.
1917-1927	946	49.9 ± 0.59	359	50.1 ± 0.97	587	49.7 ± 0.65
1928-1937	2,004	53.5 ± 0.37	719	53.4 ± 0.62	1,285	53.5 ± 0.46
Total	2,950	52.3 ± 0.32	1,078	52.3 ± 0.56	1,872	52.3 ± 0.38

* Three women of "unknown age" were excluded.
† Fifteen men of "unknown age" were excluded.

an increase in the incidence of carcinoma of the lung occurred. Were the increase in pulmonary tumors at autopsy dependent on the zeal of the hospital staff in obtaining permission for necropsy because of difficulties in diagnosis, it appears that a similar increase in tumors of the liver and bile ducts should also have been observed.

3. *Increase in the Span of Human Life.*—An analysis of the ages of persons over 20 who have come to autopsy reveals an increase in the mean age of both males and females in the period from 1928 to 1937 as contrasted with the period from 1917 to 1927. This is shown in table 3. It will be recalled that the first hundred carcinomas were noted during the period from 1917 to 1926, which nearly coincides with the first decade of this review. The mean age of men increased from 49.7 ± 0.65 years in the first of these two decades to the significantly higher level of 53.5 ± 0.46 years in the second decade. At the same time the mean age of women showed a significant increase from 50.1 ± 0.97 years to 53.4 ± 0.62 years. Men and women had the same mean age in the period from 1917 to 1927, and again the same mean age, but at a higher level, in the period from 1928 to 1937.

The "increase in the span of human life" is thus reflected in our own autopsy experience. Nevertheless, the incidence of carcinoma when standardized for age and sex failed to reveal any increase. Tables 4 and 5 illustrate this finding. Of interest is the fact that the incidence of carcinoma in each age group and in both sexes was generally higher in the period from 1917 to 1927 than in the period from 1928 to 1937. The outstanding exceptions occurred among women aged 70 and over,

TABLE 4.—*Incidence of Carcinoma Among Women Over Twenty Years of Age*

Age	1917-1937			1917-1927			1928-1937		
	Number	No. with Carcinoma	Percentage	Number	No. with Carcinoma	Percentage	Number	No. with Carcinoma	Percentage
20-29	148	2	1.4	67	0	0	81	2	2.5
30-39	184	15	8.2	55	6	10.9	129	9	7.0
40-49	193	26	13.5	62	9	14.5	131	17	13.0
50-59	217	43	19.8	56	18	32.1	161	25	15.6
60-69	183	37	20.2	61	16	26.2	122	21	17.2
70 plus	153	30	19.6	58	10	17.2	95	20	21.1
Unknown	3	0	0	2	0	0	1	0	0
Total	1,081*	153	14.2	361	59	12.4	720	94	13.1

* One woman under 20 with carcinoma was excluded from this analysis.

TABLE 5.—*Incidence of Carcinoma Among Men Over Twenty Years of Age*

Age	1917-1937			1917-1927			1928-1937		
	Number	No. with Carcinoma	Percentage	Number	No. with Carcinoma	Percentage	Number	No. with Carcinoma	Percentage
20-29	189	1	0.5	69	0	0	120	1	0.8
30-39	228	10	4.4	103	8	7.7	125	2	1.6
40-49	419	45	10.7	139	15	10.8	280	30	10.7
50-59	413	81	19.6	117	25	21.4	296	56	19.0
60-69	372	74	19.9	100	15	15.0	272	59	21.7
70 plus	251	59	23.5	59	10	16.9	192	49	25.5
Unknown	15	1	6.7	8	1	12.5	7	0	0
Total	1,887	271	14.4	595	74	12.4	1,292	197	15.2

and among men aged 60 and over; in these age groups the incidence of cancer was *greater* in the period from 1928 to 1937 than in the previous ten year experience. The combined values for the incidence of carcinoma in persons coming to autopsy, however, revealed no significant difference between the sex groups or between the two ten year periods of the survey. The incidence of carcinoma among persons over 20 years of age was 14.3 per cent, time and sex having exerted no significant influence on this value.

The material has been subjected to a further analysis with a view to ascertaining what effect, if any, the increased span of life had on the

age of persons with carcinoma at autopsy. Table 6 shows the mean age of men and of women with carcinoma in the two ten year periods of the review. The mean age of men for the twenty year period was 60.48 ± 0.69 years, which is significantly higher than the comparable age, 57.47 ± 1.10 years, for women (D = 3.01 ± 1.29; T = 2.34; P = 0.02 —). The age of men dying with carcinoma in the period from 1917 to 1927, the age of women dying in the same period and the age of women dying in the period from 1928 to 1937 did not differ signifi-

TABLE 6.—*Mean Age of Persons with Carcinoma Coming to Autopsy in the Period from 1917 to 1937*

	Total		Women		Men	
Period	Number	Mean Age, Yr.	Number	Mean Age, Yr.	Number	Mean Age, Yr.
1917-1927	132	56.8 ± 0.85	59	57.9 ± 1.20	73*	56.0 ± 1.41
1928-1937	292	60.3 ± 0.76	95	57.2 ± 1.48	197	61.8 ± 0.85
1917-1937	424	59.2 ± 0.60	154	57.5 ± 1.10	270	60.5 ± 0.69

* One man of "unknown age" was excluded.

cantly, but all three of these values were significantly lower than the mean age of men dying with carcinoma in the period from 1928 to 1937. This difference is principally the result of the unusually high age at death of the group with prostatic carcinoma, 27 of the 32 prostatic carcinomas having been observed in the period from 1928 to 1937. If men with carcinoma of the prostate are excluded in calculating the mean age of the male group, the age at death of both men and women in each of the two decades shows no significant deviation from the grand mean of 58.4 years.

TABLE 7.—*Mean Age at Death of Persons with Carcinoma at Various Major Sites*

Site	Number	Mean Age, Yr.
Prostate	32	71.5 ± 1.90
Liver and bile ducts	26	62.7 ± 2.19
Esophagus	27	61.1 ± 1.63
Rectum and sigmoid	52	57.8 ± 1.55
Stomach	58	57.7 ± 1.41
Lung	43	55.0 ± 1.58
Uterus	29	52.2 ± 1.90

The high age of the prostatic carcinoma group is shown in table 7, which gives the mean age at death of persons with carcinoma at various major sites. With the exception of the uterine carcinoma group, the pulmonary cancer group has the lowest mean age (54.95 ± 1.58 years). This was significantly lower than the mean ages of the groups with carcinoma of the prostate, liver and bile ducts, and esophagus, respectively, but did not differ significantly from the mean ages of the groups dying with cancer of the stomach, rectum and uterus, respectively.

The analysis up to this point has revealed that there was a shift to a higher level in the age at death of the general autopsy population in the period from 1928 to 1937 and that both males and females participated in this shift. There was no resultant difference in the incidence of carcinoma in either sex, and further, with carcinoma of the prostate excluded, the age at death of persons with carcinoma was essentially the same for both sexes and for each decade of the survey. If it can be shown that the frequency of carcinoma of the lung increases with increasing age, the demonstrated rise in the incidence of this neoplasm in the present survey might be explained on the basis of the general shift in age of the autopsy population to a higher level. That this shift to a higher age level during the second decennial of this survey did not

TABLE 8.—*Incidence of Carcinoma of the Lung Among Men—Observed and Expected Values*

	1917-1937			1917-1927			1928-1937		
		Number with Carcinoma of Lung			Number with Carcinoma of Lung			Number with Carcinoma of Lung	
Age	Men		Percentage	Men	Observed	Expected	Men	Observed	Expected
20-29	189	0	0	69	0	0	120	0	0
30-39	228	2	0.88	108	1	0.9	125	1	1.1
40-49	419	10	2.39	139	2	3.3	280	8	6.7
50-59	413	14	3.39	117	4	4.0	296	10	10.0
60-69	372	9	2.42	100	1	2.4	272	8	6.6
70-79	198	3	1.20	42	0	0.7	151	3	2.3
80-89	51	0	0	13	0	0	38	0	0
90-99	7	0	0	4	0	0	3	0	0
Unknown	15	0	0	8	0	0	7	0	0
Total	1,887	38	2.01	595	8	11.3	1,292	30	26.7

account for the observed increase in the frequency of pulmonary cancers is demonstrated in table 8, which is based on the age distribution of 38 pulmonary carcinomas among men (5 among women are excluded) related to the male autopsy population in each age group. It will be noted that the incidence of carcinoma of the lung rose gradually to its highest value of 3.39 per cent in men 50 to 59 years of age and then fell to 1.20 per cent in men aged 70 to 79; no pulmonary carcinomas were observed in 58 men older than 79 years. From these values obtained from the entire twenty year period of the survey, the number of pulmonary cancers in men was estimated for the period from 1917 to 1927 and for that from 1928 to 1937. The estimated values did not differ significantly from the observed numbers.

In this connection it is of interest to note a somewhat different finding as regards carcinoma of the prostate (table 9). Here the precipitous increase in incidence beginning at the age of 80 to 89 is

striking. Prostatic carcinoma was noted in 9 of the 58 men over 79 years, in contrast to cancer of the lung, which did not occur in any of the men comprising this age group. This finding confirms the observations of Rich [11] and of Moore,[12] who noted an increasing incidence of prostatic carcinoma with increasing age. It is conceivable, therefore, that a further increase in life span might result in a future marked increase in prostatic malignant tumors. With regard to the present survey, however, it is apparent that the increased incidence of pulmonary carcinoma was not influenced by the demonstrated increased span of life.

4. *No Comparable Increase in Tumors of the Skin.*—Frissell and Knox stated that "since external and readily diagnosed cancer has not increased, it would seem probable that . . . the increase in the

TABLE 9.—*Incidence of Carcinoma of Prostate—Observed and Expected Values*

	1917-1937			1917-1927			1928-1937		
		Number with Carcinoma of Prostate			Number with Carcinoma of Prostate			Number with Carcinoma of Prostate	
Age	Men		Percentage	Men	Observed	Expected	Men	Observed	Expected
20-29	189	0	0	69	0	0	120	0	0
30-39	228	0	0	103	0	0	125	0	0
40-49	419	1	0.24	139	0	0.3	280	1	0.7
50-59	413	3	0.72	117	1	0.8	296	2	2.1
60-69	372	12	3.22	100	1	3.2	272	11	8.8
70-79	193	7	3.63	42	1	1.5	151	6	5.5
80-89	51	7	13.73	13	1	1.8	38	6	5.2
90-99	7	2	28.57	4	1	1.1	3	1	0.9
Unknown	15	0	0	8	0	0	7	0	0
Total	1,887	32	1.7	595	5	8.7	1,292	27	23.2

incidence of bronchial carcinoma in the last two decades is apparent rather than real." This point has also been made by Fried: "It is remarkable that parallel investigations on the incidence of external . . . cancers show no increase." The statement that an increase in tumors of the skin has not occurred is not indisputable. Mackee and Cipollaro [13] reported that, "of 76,274 deaths caused by malignant neoplasms in the registration area of the United States in 1921, 2,610 (3.42 per cent) were due to cancer of the mouth. . . . Malignancy of the skin was given as the cause of death in 2,433 cases (3.19 per cent). In 1934 the corresponding figures were 134,428 cancer deaths, 5,009 due to cancers of the mouth . . . and 3,315 due to cancers of the

11. Rich, A. R.: J. Urol. **33**:215, 1935.

12. Moore, R. A.: J. Urol. **33**:224, 1935.

13. Mackee, G. M., and Cipollaro, A. C.: Cutaneous Cancer and Precancer, New York, American Journal of Cancer, 1937.

skin. Thus during a period of thirteen years death from cancer of the buccal cavity and skin showed an increase of 3,280." However, even if there were convincing evidence that the incidence of cutaneous tumors has not increased, there is no logical reason for assuming a corresponding constancy in the incidence of malignant growths of any of the visceral organs. In the opinion of Peller,[14] an inverse relationship exists between cancer of the skin and lip and cancer of other sites. This, however, has been seriously questioned in a recent report by Conrad and Hill,[15] who found on the average a slight direct association, occupational groups with a relatively high rate of cancer of the skin and lip tending to show also an excess of cancers of other sites. In any event, there is no available evidence to indicate a significant relationship between cancer of the skin and lip and carcinoma of the lung.

To what extent are conclusions drawn from the numbers of persons coming to autopsy applicable to the general population? This question cannot be answered categorically. Although autopsy experience is based on a relatively small proportion of the general population, pathologic diagnoses are admittedly more accurate than clinical ones. Whether or not in the problem at hand accuracy in diagnosis is sufficient to compensate for deficiency in size of sample is not known. Certain evidence bearing on this problem is, however, worthy of mention.

Hirsche[16] studied 1,700 death certificates representing the total mortality from cancer in resident and nonresident citizens of New Haven during the seven year period from 1925 to 1931. It was observed that 803, or 47 per cent, of the deaths occurred in the three main hospitals of New Haven, and that 237, or 14 per cent, of the deaths took place in the New Haven Hospital. Thus 1 of every 7 persons whose death certificates bore a diagnosis of cancer in the period of Hirsche's review, died in this hospital. Although the number of necropsies performed on this group is not known, the size of the sample is sufficiently large to permit tentative conclusions concerning the population from which the sample was derived.

A second study by Hirsche[17] indicates the inaccuracies of cancer death certificates, both as to content and as to total number of deaths from cancer. This investigator examined the death certificates and clinical diagnoses of 217 persons dying of malignant disease and examined post mortem in the department of pathology at Yale University

14. Peller, S.: Lancet **2**:552, 1936.

15. Conrad, K. K., and Hill, A. B.: Am. J. Cancer **36**:83, 1939.

16. Hirsche, H. F.: A Study of Cancer Deaths, in The Cancer Problem in New Haven: Report of the Cancer Committee of New Haven, 1935, appendix C.

17. Hirsche, H. F.: A Comparative Study of Post Mortem Diagnoses, in The Cancer Problem in New Haven: Report of the Cancer Committee of New Haven, 1935, appendix B.

School of Medicine between Jan. 1, 1925 and Dec. 12, 1933. The diagnosis given in the death certificate tallied with the cause of death as determined at necropsy in 157, or 72 per cent, of these cases. In 25 instances, or 12 per cent, of the total, no mention of a malignant tumor was made on the death certificate, and in 35 cases, or 16 per cent, the primary site of the malignant growth as recorded on the death certificate did not correspond with that observed at necropsy. It should be emphasized that these discrepancies occurred on certificates which should have been correct in every instance.

The greatest errors occurred among cases of primary pulmonary tumor. Nineteen such cases were included in the survey, and in 11, or more than half, the clinical diagnosis was incorrect. Tuberculosis was clinically diagnosed in 3, and each of the following diagnoses was made in 1 instance: carcinoma of the esophagus, carcinoma of the stomach,

TABLE 10.—*Comparison of Cancer Mortality in Persons Twenty Years of Age and Older in the State of Connecticut and in the Series Coming to Autopsy at Yale University in the Period from 1918 to 1937*

		State of Connecticut	Yale Autopsies
1918-1927	Deaths in general *	139,496	956
	Deaths from cancer	14,668	133
	Percentage	10.51	13.91
1928-1937	Deaths in general *	149,358	2,012
	Deaths from cancer	20,293	291
	Percentage	13.58	14.46

* Deaths of persons over 20 years of age.

pleurisy, hypernephroma, neoplasm in centrum ovale, lymphosarcoma and heart disease; in 1 instance the condition was not determined. The death certificate in 8 of these 19 cases was incorrect. The highest correlation between clinical diagnoses and observations at necropsy was found in the cases of gastric carcinoma. An incorrect clinical diagnosis had been made in only 2 of 32 such cases, while in 5 the death certificate was in error.

On the basis of the foregoing studies it appears, first, that approximately 1 of 7 local patients whose death certificates bear a diagnosis of malignant tumor died at the New Haven Hospital, and, second, that gross inaccuracies occur in both clinical diagnoses and death certificates, carcinoma of the lung being the most frequently misdiagnosed malignant tumor. Since the data on the mortality from cancer are characterized by errors both of omission and of commission, as indicated by the death certificates, it is of interest to compare the cancer death rates in the two decennials of the present survey with the corresponding values for the state of Connecticut, these rates being based on the total numbers of

persons over 20 years of age. Table 10 indicates a mortality of 13.91 per cent from cancer in the reported material in the years 1917 to 1927 as compared with a mortality of 10.51 per cent in the entire state. The next decennial, 1928 to 1937, showed an increase in both series, the value in our own survey being 14.46 per cent, and that in the state, 13.58 per cent. If due allowance is made for the greater accuracy of the data recorded from autopsies over those in death certificates, as indicated by Hirsche's study, the discrepancy between the cancer mortality in our autopsy population and that in the state at large would be reduced considerably.

In 1920, following an extensive experience with the pathologic aspects of influenza, Winternitz, Wason and McNamara [18] stated that "an increase in primary carcinoma [of the lung] would probably occur later" because of "metaplasia of the bronchial epithelium causing a proliferation of the young cells." The great majority of studies since then have demonstrated the prophesied increase, and although influenza has not been proved definitely to be the factor involved, it nevertheless plays an important role in all discussions of the causes. The present report has analyzed certain factors which have frequently been cited to substantiate the belief that this increase is only relative. These factors have been shown to have exerted little or no influence on the statistically significant increase in primary carcinoma of the lung found at autopsy in the New Haven Hospital, and it is concluded that the observed increase is real and absolute. Indirect evidence appears to justify the additional conclusion that the real increase in primary carcinoma of the lung observed in our autopsy material is characteristic also of the population at large.

SUMMARY

The 4,156 autopsies performed at Yale University School of Medicine between Sept. 22, 1917 and Dec. 31, 1937 have been reviewed with a view to determining the incidence of carcinoma in general and of carcinoma of the lung in particular. The ratio of pulmonary carcinomas to all carcinomas increased from 7.4 per cent during the decade from 1917 to 1927 to 11.0 per cent during that from 1928 to 1937.

A method for the calculation of regression equations employing equal numbers of carcinomas as the independent variable instead of equal intervals of time is here introduced. The regression equation for carcinoma of the lung calculated on this basis showed a significant positive deviation from zero during the period under review. None of the

18. Winternitz, M. C.; Wason, I. M., and McNamara, F. O.: The Pathology of Influenza, New Haven, Conn., Yale University Press, 1920.

regression equations calculated in a similar manner for carcinoma of the stomach, liver and bile ducts, prostate, intestine, female genitalia, esophagus and rectum showed a significant deviation from the horizontal.

The factors which might have been responsible for the observed increase in the incidence of primary carcinoma of the lung were analyzed. It was found that the general autopsy population of persons over 20 shifted to a higher age level during the period from 1928 to 1937 as compared with the previous decade. This shift, however, did not account for the observed increase in the incidence of primary pulmonary tumors.

It was concluded that the increase in pulmonary cancers observed in our autopsy material was a real and absolute increase. Indirect evidence appeared to indicate that this conclusion was also applicable to the general population.

DISSECTING ANEURYSM OF THE AORTA IN EXPERIMENTAL ATHEROSCLEROSIS

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There is a growing interest in the study of dissecting aneurysm of the aorta. This has been stimulated mainly by the successful crystallization of knowledge of the clinical features of the disease. The condition is being more frequently recognized at the bedside rather than at the autopsy table. The incidence of the condition appears to be increasing. Thus in a group of 3,206 necropsies in the Boston City Hospital during the years from 1927 to 1933, inclusive, dissecting aneurysms were found in 1 case out of 320,[1] while in a group of 2,202 necropsies from 1935 to 1937, inclusive, the proportion was 1 to 200.[2] During the same period (1935-1937) syphilitic aneurysms of the aorta occurred in 1 case out of 160. A comparison of the frequency ratios of the two conditions suggests that, with the expected future decrease of syphilis and increase of vascular degenerative disease, dissecting aneurysm may eventually become more frequent than syphilitic aneurysm. Thus dissecting aneurysm is more than a clinical curiosity.

In the genesis of dissecting aneurysm medionecrosis aortae is the usual predisposing factor, with hypertension the common activating agency. Atherosclerosis is responsible for a certain percentage of cases, and rheumatic aortitis is said to be a possible cause. Physical or mental disturbances may be contributing agents in the actual rupture, probably because of their influence on hypertension, and mechanical injury (external or internal trauma) has been reported as an exciting element.

EXPERIMENTAL DISSECTING ANEURYSM

Before discussing experimental lesions it is necessary to consider the possibility of the occurrence of spontaneous dissecting aneurysm in the rabbit. Personal experience and special inquiries in laboratories, veterinary schools and zoos have failed to reveal cases. Liebig [3] made a

This investigation was aided by a grant from the Committee on Scientific Research of the American Medical Association.

1. Weiss, S.: M. Clin. North America **18**:1117, 1935.
2. Weiss, S.: New England J. Med. **218**:512, 1938.
3. Liebig, H.: Arch. f. exper. Path. u. Pharmakol. **159**:359, 1931.

definite statement that spontaneous aneurysm of the aorta of the rabbit is unknown.

Bennecke,[4] in a study of lesions of vessels following the use of poisons, recorded his findings in 400 rabbits that were not subjected to experimentation but died spontaneously or from intercurrent infections. He pictured without description an aortic malformation observed in a rabbit which may have been a short dissecting aneurysm, and also an irregular dilatation of the aorta of another of these animals. In the literature on the subject which he discussed there was no reference to similar lesions, and no publications since that time have recorded such changes in normal rabbits.

MEDIAL NECROSIS IN THE RABBIT

The media of the aorta of the rabbit is not infrequently the seat of minute lesions. These are sharply localized and consist of degenerative and necrotic processes with a strong tendency toward calcification. Miles and Johnstone [5] and Levin and Larkin [6] described lesions, practically all of which were minute, in 35 to 52 per cent of normal rabbits. These lesions were limited, almost without exception, to the media or were of medial origin.

EPINEPHRINE-INDUCED NECROSIS IN THE MEDIA OF THE RABBIT AORTA

The discovery of medial lesions in the aorta of the normal rabbit was used to discountenance claims that aortic lesions following injections of epinephrine hydrochloride were due to that drug. Lesions produced in the aorta of the rabbit by the vasopressor effects of epinephrine, however, are more extensive than the minor processes found in normal rabbits. Josué [7] first reported the effects of injections of epinephrine in 1903. The resemblance of these lesions to those of medionecrosis aortae in the human aorta is close. As would be expected, some dissecting aneurysms occurred in these experimental animals.

Fisher [8] described a dissecting aneurysm extending from the arch of the aorta to the level of the renal arteries in an animal in which paralysis of both hindlegs developed following the twenty-third injection of epinephrine. He emphasized the fact that the lesions of the aorta following repeated injections of epinephrine are a "primary necrosis of the muscular and elastic layers of the media" and that these lesions have little in common with the changes observed in atherosclerosis of the human aorta.

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4. Bennecke, A.: Virchows Arch. f. path. Anat. **191**:208, 1908.
5. Miles, A. B., and Johnstone, O. P.: J. A. M. A. **49**:1173, 1907.
6. Levin, I., and Larkin, J. H.: J. Exper. Med. **13**:24, 1911.
7. Josué, O.: Compt. rend. Soc. de biol. **55**:1374, 1903.
8. Fisher, B.: Deutsche med. Wchnschr. **31**:1713, 1905.

Erb [9] described among a large group of experimental animals a young rabbit, weighing 1.3 Kg., which received over a period of about two months thirty-one injections of 0.1 to 0.3 cc. of a 1:1,000 solution of epinephrine hydrochloride. A few minutes after the last injection flaccid paralysis of the hindlegs and severe dyspnea developed. The animal died six hours later. Necropsy revealed hemoperitoneum, with clots, and retroperitoneal hemorrhage about the aorta. Below the renal arteries the aorta was collapsed and white. There was serosanguinous fluid in both pleural cavities as well as hemorrhage into the tissues about the thoracic portion of the aorta. There was a dissecting aneurysm originating in the lower part of the arch and extending to the level of the renal artery. This started between the intima and the media and continued into the media and adventitia.

In Külbs's [10] series 1 rabbit received thirty-one injections of massive doses of epinephrine in sixty-two days. Death acutely followed the last injection. Necropsy revealed hemorrhages into both pleural cavities. The mediastinal tissues were infiltrated with blood. There was an intimal tear, the location of which was not mentioned. The dissection extended downward to the iliac junction. On microscopic examination there was separation of the intima and media at the site of the original rupture. Elsewhere the adventitia was elevated by hemorrhagic dissection.

Among Ziegler's [11] animals a dissecting aneurysm developed in 1 rabbit. An accidental fall from a height of 1.5 meters precipitated the rabbit's death. This animal, 8 weeks old, had received seven injections of 0.15 cc. of a 1:1,000 solution of epinephrine hydrochloride. At postmortem examination there was hemorrhagic infiltration of the periaortic tissues with a diffuse hematoma of the aortic wall. There was an intimal tear through which blood penetrated between the intima and the media. Below this the layers of the diseased media were separated, and the adventitia had ruptured at the level of the diaphragm.

Kaiserling [12] reported 1 animal in his series with dissecting aneurysm. This rabbit received thirty-eight doses of a 1:1,000 solution of epinephrine hydrochloride, varying from 0.1 to 0.7 cc., over a period of forty-four days. Several hours after the last dose, 0.7 cc., the animal showed sudden paralysis of the hindlegs, dyspnea and rapid pulse. It became apathetic and was found dead the next morning. Necropsy disclosed two tears in the aortic intima. One, 2 cm. long, was in the thoracic aorta with separation and rupture of the adventitia. The second small tear was located immediately below the diaphragm. Here the dissection had

9. Erb, W.: Arch. f. exper. Path. u. Pharmakol. **53**:173, 1905.
10. Külbs: Arch. f. exper. Path. u. Pharmakol. **53**:140, 1905.
11. Ziegler, K.: Beitr. z. path. Anat. u. z. allg. Path. **38**:229, 1905.
12. Kaiserling, K.: Klin. Wchnschr. **44**:29, 1907.

torn an artery. It is of interest that histologic examination failed to reveal any localized lesion of the aortic media.

Schirokogoroff [13] reported dissecting aneurysms in 2 rabbits of an epinephrine series. A rabbit 3½ months old died fifteen hours after the last injection. The animal had received seventeen injections of 0.15 cc. of a 1:1,000 solution of epinephrine hydrochloride. The record of the postmortem examination lacks details, but it is stated that a dissecting aneurysm was present and that there was necrosis of the muscle and elastic fibers of the aortic media. The second rabbit had received forty injections of 0.15 cc. of a 1:1,000 solution of epinephrine in three months and four days. In the record of the postmortem observations there is mention merely of the presence of a dissecting aneurysm and necrosis of the muscle layer of the media.

These reports of aortic dissecting aneurysm following injections of epinephrine are in large part lacking in detail. The aneurysms are casually recorded as interesting observations in the course of experiments dealing primarily with the general effects of the drug. The thickness of the human aortic media favors the dissecting apart of its layers by the blood as it spreads. The thin media in the aorta of the rabbit does not lend itself so readily to separation of the fibers, so that in most cases the dissection tends ultimately to occur between the media and the adventitia.

LESIONS INDUCED IN THE AORTA OF THE RABBIT BY VITAMIN D

The feeding to rabbits of massive doses of viosterol or of other preparations of vitamin D gives rise to medial necrosis with a strong tendency toward calcification. Dissecting aneurysm of the aorta was not reported as occurring in these animals. The lacking element in these experiments was probably the vasopressor factor.

ATHEROSCLEROTIC (INTIMAL) LESIONS IN NORMAL RABBITS

It is apparent that the lesions found in normal rabbits and those produced by epinephrine or by vitamin D are limited to, or arise from, the media.

Clarkson and Newburgh [14] cited records of 2,947 rabbits reported by several workers in experimental medicine with ten possible examples (0.34 per cent) of spontaneous sclerosis of the aorta which might be of the atherosclerotic type. Atherosclerosis can be produced, however, in 100 per cent of rabbits by feeding them adequate amounts of cholesterol, and the lesions will cover much of the surface of the aorta

13. Schirokogoroff, J. J.: Virchows Arch. f. path. Anat. **191**:482, 1908.
14. Clarkson, J., and Newburgh, L. H.: Arch. Int. Med. **31**:653, 1923.

Fig. 1.—Dissecting aneurysm of the aorta of a rabbit (see text for a full description).

in contrast to the relatively minute lesions described so rarely in normal rabbits. There can be no confusion, therefore, between the rare processes found in normal animals and the constant results of adequate cholesterol feeding.

REPORT OF A CASE

A male rabbit had been fed a total of 60 Gm. of cholesterol in solution in sunflower seed oil by catheter. There were 110 feedings from Oct. 22, 1934 to May 7, 1935. The weight of the rabbit increased during that period from 1,780 to 2,880 Gm. Feeding had progressively increased from 0.3 Gm. of cholesterol daily six times a week to 0.9 Gm. in January 1935. Because of an epidemic among the rabbits, feeding was stopped Jan. 26, 1935; it was resumed March 8 (0.3 Gm.) and continued until May 7.

The animal was allowed to live after cessation of the cholesterol feeding. During the three years intervening before its death it was kept on a diet of rabbit chow.[14a] When last seen alive, April 12, 1938, the animal was apparently well. Its weight had been standardized at about 3,400 Gm. for some time before death.

On the morning of April 13, 1938 the animal was found dead in its cage. At postmortem examination the right pleural cavity contained currant jelly clots and a small amount of fluid blood. The left pleural cavity contained a small amount of clot and fluid blood. There was hemorrhagic infiltration of the periaortic tissues downward to the level of the diaphragm. There was hemorrhagic infiltration of the tissues about the esophagus up to the base of the skull. Rupture into the pleural cavity on the right had occurred through a slitlike opening in the pleura just above the diaphragm. The point of rupture into the left pleural cavity could not be found. The aorta was tortuous, the wall was thickened and opaque, and there was dilatation of the arch and upper thoracic portion. When the vessel was laid open, an oval ulceration 0.5 by 0.3 cm., was seen in the anterior wall, just below the junction of descending arch and thoracic portion. This was lined by a granular red membrane. The layer of blood, located between the deep layers of the vessel wall, extended upward along the aorta to the level of the orifice of the left subclavian artery and downward almost to the diaphragm (fig. 1). It varied in thickness up to 0.9 cm. Calcification of the wall was continuous in the lower thoracic region, producing a waistlike effect. The abdominal portion of the vessel was dilated, but the wall was less markedly thickened than in the thoracic portion. The aortic intima was thickened, nodular and colored brownish yellow, with yellow predominating in the nodules. The nodular and diffuse thickening of the intima was continuous down to the midabdominal region. Below this level were scattered yellow nodules. The vessels of the arch showed marked thickening of their walls with great narrowing of their lumens, that of the right carotid artery being almost occluded. The walls of the carotid arteries showed small patches of yellow opaque thickening to within 1 cm. of the base of the skull.

The heart was hypertrophied; the cavities were empty. The left and right coronary arteries showed thickened walls and narrowed lumens.

The right lung was compressed and airless except over a narrow region along the anterior edge. The left lung had compression atelectasis over the back of the lower lobe but was otherwise normal. The bronchi and trachea were natural. The liver was of normal size and a gray-red color, with focal regions of irregular lobulation and scarring. The gallbladder contained an estimated 3 cc. of bluish

14a. This chow is a standard stock feed containing all necessary food substances but no animal cholesterol as such.

black bile. The mucosa was natural. In the middle portion of the spleen was a firm subcapsular nodule, 0.7 cm. in greatest diameter, which on section was opaque yellowish white. Apart from this nodule the organ was normal.

The left kidney was of normal size; the right, smaller. The capsules peeled with little difficulty. The surface of the right kidney showed a series of depressed scars, beneath which, on section, the cortex was thinned. The surface of the left kidney was smooth. The cut surfaces showed bilaterally a series of opaque yellow streaks running from the intermediate zone toward the apexes of the pyramids.

The adrenals, though of normal size, were softer than normal. On section there was a suggestion of thickening of the cortex. The testicles were of normal appearance, and the thyroid, the brain and cerebral arteries and the gastrointestinal tract were likewise normal. There was no gross evidence of cholesterol deposits in the eyes or in the skin.

Microscopic Examination.—(*a*) Aorta: Near the site of the atheromatous ulcer the aortic wall showed, together with thinning and hyaline degeneration of the media, a markedly thickened intima made up largely of hyaline connective tissue. In the base of the ulcer, at one side the intima was almost wholly replaced by a richly cellular tissue, made up of young fibroblasts paralleling one another, with their long diameters at right angles to the media or remaining deep fragments of the intima. This young granulation tissue was diffusely infiltrated with lymphoid and plasma cells, macrophages and a few polymorphonuclear leukocytes. In places the continuity of the intima was broken and the granulation tissue was based on the hyaline remnants of the media. In the midregion of the ulcer was a hyaline fibrinous thrombus undergoing organization which in places where the media was absent rested directly on the adventitia. The general picture was that of deep necrosis of the thickened intima and in part of the media, followed by thrombosis and progressing organization of the clot (fig. 2 *A*).

Alongside the ulcer a hemorrhage into the wall could be seen at one point, separating the layers of the media (fig. 2 *B*), but for the most part the clot lay between the media and the adventitia as it spread up and down the aorta.

The aortic wall in general owed its thickening largely to an intimal process. It was made up in great part of a connective tissue varying in density from that of moderately cellular collagenous tissue to dense, relatively acellular, hyaline connective tissue (fig. 3 *A*). The surface layer was loose textured and showed infiltration with lipoid foam cells and lymphocytes. In sections stained for fat the middle and deep layers of the intimal fibrous tissue contained abundant isotropic granular lipoid.

(*b*) Heart: The muscle fibers were hypertrophied. In the main coronary arteries the intima was thickened by hyaline fibrosis, and the lumens were narrowed. In some of the muscular branches the narrowing of the lumens was marked (fig. 3 *B*), and one smaller branch showed complete occlusion. At the summit of some of the muscle folds, beneath the endocardium the muscle fibers were shrunken, distorted and in part replaced by relatively acellular connective tissue (fig. 4 *A*).

(*c*) Carotid Arteries: The intima of the right carotid artery (fig. 4 *B*) was thickened by a growth of connective tissue, which had narrowed the lumen to an angular slitlike opening. In a deeper crescentic fold a hyaline thrombus was undergoing organization, and the inner layer of the intima was made up of young fibroblastic tissue infiltrated with lymphoid cells and monocytes. In the other folds the endothelial layer lay on a relatively loose-textured connective tissue, which made up much of the inner layer of the intima. Near the media there was an encircling layer of dense hyaline connective tissue, thrown into fluted folds, internal

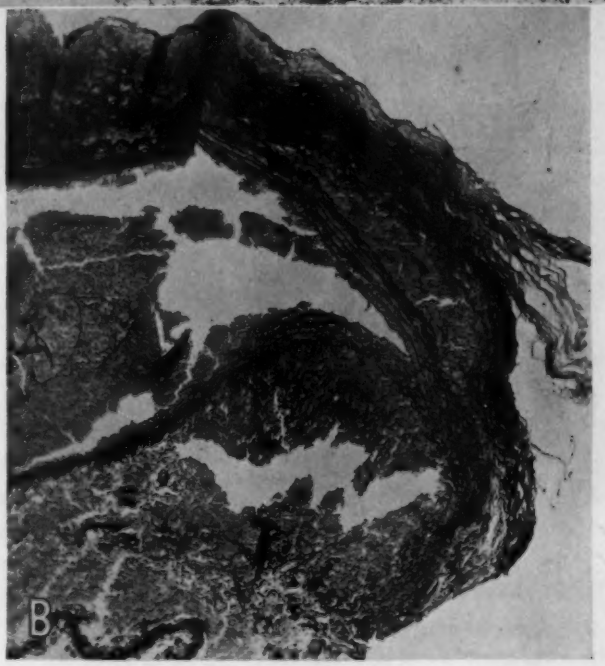

Fig. 2.—*A*, atheromatous ulcer with thrombosis (see text). *B*, blood dissecting apart the layers of the aortic media near the ulcer.

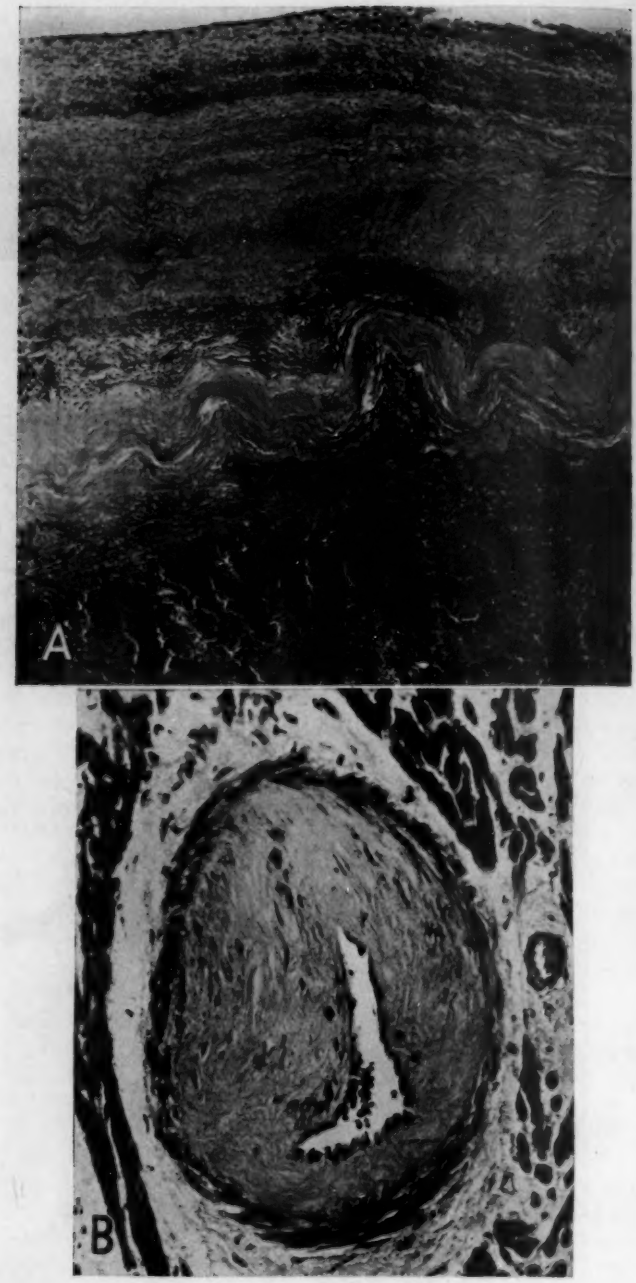

Fig. 3.—*A*, aortic atherosclerosis. The thickening of the intima is largely due to fibrosis and hyalinization of the fibrous tissue. Below is a mass of blood outside the media, actually separating the media from the adventitia.

B, fibrous narrowing of the lumen of a coronary branch—an old healed lesion.

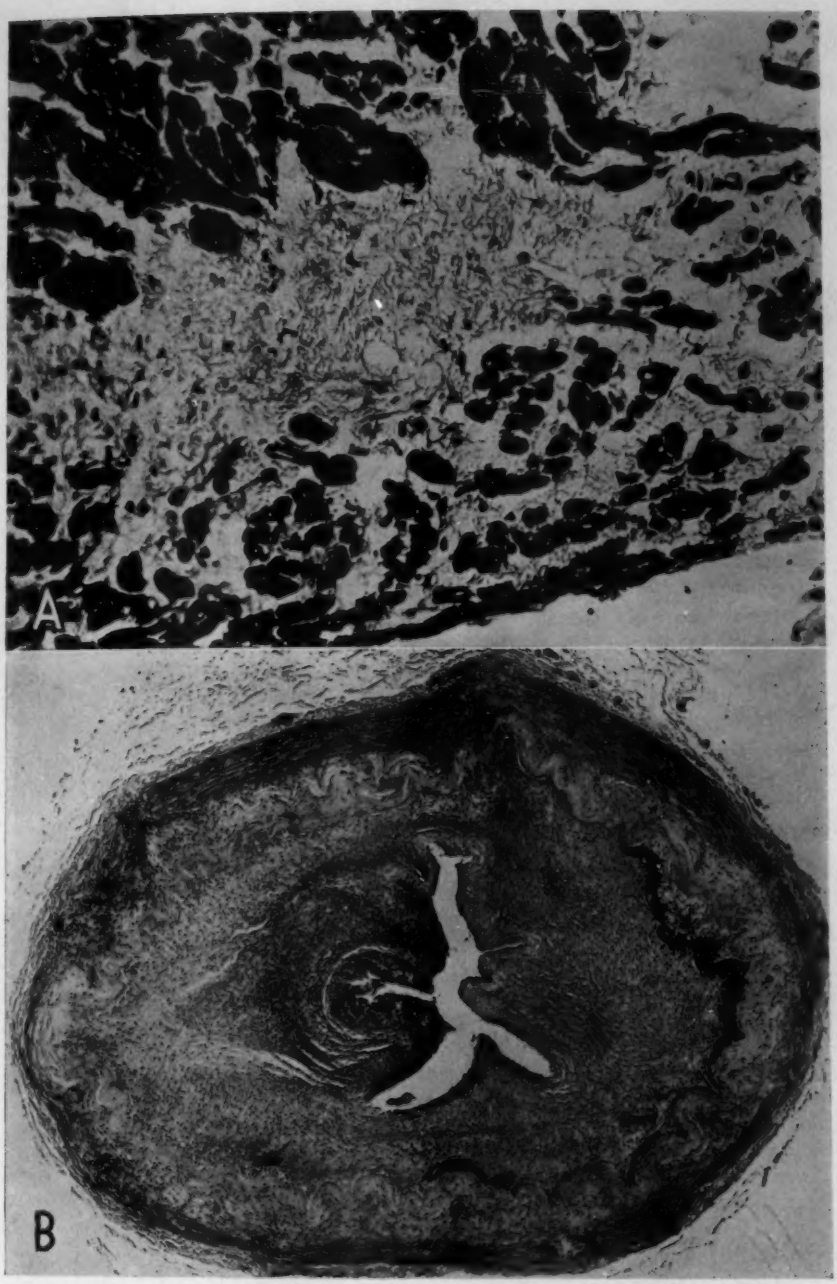

Fig. 4.—*A*, old repaired atrophy and necrosis of cardiac muscle fibers (chronic vascular myocarditis). Compare the size of the fibers to the right and below with those to the left and above. *B*, section of the right carotid artery about 0.5 cm. from its origin (see text).

to which in places was an acellular material staining pink with eosin. (In frozen sections stained for fat this material and the dense hyaline layer surrounding it were rich in sudan-staining granular lipoid. Under the polariscope some anisotropic crystals were scattered through the fatty material.) The media showed irregular thinning, and in places only a few fibers remained. At points where vasa vasorum were penetrating through the media there was some infiltration with lymphoid cells, and the adventitia about these regions showed lipoid cell infiltration. In frozen sections stained with sudan IV there were lipoid cells scattered in places in the media. Sections stained by Verhoeff's or Weigert's elastic stains disclosed irregularity, fragmentation and disappearance of the elastica in the media. In the fluted hyaline layer there was an irregular production of elastic tissue in the form of series of fine fibrils, with a heavier elastic band along the fluted edge. This inner elastic lamina could be followed for some distance as an intact membrane and then was seen to become granular and disappear in the fatty detritus in this region.

The left carotid and the subclavian arteries were the seat of similar lesions, the right subclavian artery showing a lumen so narrow that at one level the intimal folds were almost in contact.

(*d*) Liver: For the most part the organ was normal. In the regions of irregular lobulation there was an increase of periportal connective tissue outlining the lobules, many of which were distorted. There was moderate infiltration of the periportal tissue by lymphocytes.

(*e*) Spleen: The splenic tumor was poorly encapsulated and was made up of oat-shaped cells, which tended to occur in whorls, as in a meningioma. Mitoses were present. Lipoid-containing cells were few. Otherwise the spleen was not remarkable.

(*f*) Kidneys: The arteries of the right kidney showed high grade fibrous sclerosis with narrowing of the lumens, which was extreme in places. In the regions of thinning of the cortex there was diffuse fibrosis with lymphoid cell infiltration. Glomeruli were found in all stages from thickening of the capsule, through adhesion of the tufts, to complete hyaline scarring. Alongside these regions the cells lining convoluted tubules were swollen and granular, with fine granules which took the fat stain. Much of the renal structure away from the regions of fibrosis was normal, and the tubular epithelium contained no fat granules. The yellow streaks seen grossly in the pyramids were made up of collections of cholesterol crystals embedded in fibrous tissue.

The left kidney was essentially normal save for masses of cholesterol crystals shut off in dense connective tissue in streaks in the pyramids.

(*g*) Adrenals: The cortical layer was thickened and rich in cholesterol. Lying in the zona reticularis were many small collections of cells, which took a purple stain with nile blue sulfate. Similar groups of cells were present in lymph vessels in the medulla. The contents of these cells showed little or no anisotropism, a rare crystal being present in an occasional group, in contrast to the cortical cells, which were rich in cholesterol esters.

The anatomic diagnoses were exsanguination; bilateral hemothorax; dissecting aneurysm of the aorta; atheromatous ulcer of the aorta; advanced atherosclerosis of the aorta with calcification and dilatation of the arch and the thoracic portion; atherosclerosis of the carotid, of the subclavian and of the coronary arteries; hypertrophy of the heart with focal chronic vascular myocarditis; chronic vascular nephritis on the

right; embedded cholesterol crystals in renal pyramids bilaterally; focal subacute and chronic interstitial hepatitis; fibrosarcoma of the spleen.

HUMAN AND EXPERIMENTAL ATHEROSCLEROSIS

Anitschkow,[15] in his review of experimental atherosclerosis, records with respect to atherosclerotic lesions in the rabbit the following variations from human processes:

> *The principal differences between experimental cholesterin atherosclerosis in rabbits and human atherosclerosis are as follows:* (1) In experimental atherosclerosis no fatty infiltration of the elastic fibers or lamellae is ever observed, as is often the case in human patients. In rabbits, the lipoid substances generally accumulate only in the ground substance of the arterial wall and in the cellular elements of the newly formed intima. (2) In material derived from the experimental animals one finds much larger quantities of lipoidal cells than is generally the case in human material of a corresponding nature. It is true though that in human material the quantity of lipoidal cells found in the newly formed intima is subject to great variations; and in some cases it is very considerable. (3) In cholesterinized rabbits neither the hyaline swelling of the fibrous atherosclerotic plaques nor the development of fibrous elements in general is as pronounced as in human atherosclerosis. (4) In rabbits no ulcers are ever observed in the atherosclerotic plaques, whereas in human atherosclerosis such ulcers are quite frequent; they are sometimes covered with thrombotic deposits. (5) The distributions of the atherosclerotic changes in the arteries is somewhat different in rabbits, as compared with human atherosclerosis, although the arteries affected are generally the same. The cerebral arteries however—to mention one exception—are never affected in rabbits.

It is evident from the material presented in this case that most of the differences which Anitschkow cited resulted from the comparison of early experimental lesions with late human lesions. The study of a large series of rabbits which were fed cholesterol and *permitted to live for years after cholesterol feeding ceased* has demonstrated that when late rabbit lesions are compared with standard (late) human atherosclerotic lesions the differences are minor. Thus, with reference to Anitschkow's claimed differences:

1. Fatty degeneration of the elastica is a late human lesion, appearing as the process includes the media. Similarly, in late experimental lesions fatty degeneration occurs in the elastica.

2. Figures 3 and 4 *B* indicate that in late experimental lesions lipoid cells are at least no more abundant than in human lesions.

3. These illustrations also establish that in the experimental lesions hyaline changes in the connective tissue and the fibrosis in general correspond to those found in late human lesions.

4. The atheromatous ulcer in this case negatives claimed difference 4.

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15. Anitschkow, N., in Cowdry, E. V.: Arteriosclerosis: A Survey of the Problem, New York, The Macmillan Company, 1933, pp. 305-306.

5. There are differences in the localization of the aortic lesions in the experimental and the human disease. In man the ascending portion of the aorta resists the production of advanced atherosclerotic lesions. This region is a favorite site for the early deposit of lipoid cells. Crops of pinhead-sized lesions, due to invasion of the subendothelial layer of the intima by these cells, are frequently met with. These may spread in fanlike extensions over the intima but remain superficial. The cholesterol in lesions of this portion of the aorta is removed by fibroblastic cells, as one of us (T. L.) has demonstrated.[16] The lesions lose their orange color, become pale yellow, then gray, and finally disappear. The cholesterol provokes a growth of fibroblastic tissue in these lesions, but this tissue does not form collagen, so that scarring does not follow. The mechanism referred to also removes cholesterol deposits from the aorta and other vessels in the young. It is efficient, even into old age, in the ascending portion of the aorta, though it shows little or no activity in other parts of the vessel.

In the rabbit there is no resistance of the ascending portion of the aorta to the production of atherosclerosis, or else the large doses of cholesterol in the diet overwhelm the mechanism of resistance. As a result, the lesions extend continuously from the ring over the arch and thoracic portion of the aorta. In the late stages dilatation occurs throughout this region and calcification, often continuous, is usual.

The resemblance of this continuous atherosclerotic process, with its dilatation and calcification, to the atherosclerotic lesions associated with human syphilitic aortitis is close. In syphilitic aortitis the resistance of the ascending aorta to atherosclerosis is abolished, and a secondary atherosclerotic process is found—continuous from the ring and over the arch into the thoracic portion of the aorta. This is accompanied by dilatation and calcification, which is frequently continuous.

Human cerebral arteriosclerosis is usually a very late manifestation of the disease, associated with senility. No experimental rabbits have been permitted to live long enough to correspond in relative age to the senile human being with cerebral arteriosclerosis.

COMMENT

In this paper is reported the first atheromatous ulcer of record in an experimental animal and the first dissecting aneurysm in a rabbit fed cholesterol.

In the literature on the subject of dissecting aneurysms in rabbits under experimental conditions it is made clear that this lesion most frequently follows necrosis of the aortic media. In the group of animals which received repeated doses of epinephrine, severe paroxysmal arterial

16. Leary, T.: Arch. Path. **21**:419, 1936.

hypertension played the primary role in the causation of the dissecting aneurysm. It is probable that the necrotic lesions of the media resulted from the damaging effect of the hypertension. This type of pathogenesis corresponds to that often present in human subjects in whom there is an association of severe hypertension with medionecrosis but in whom the intima is normal. The significance of hypertension as a factor in the actual rupture is suggested by the failure of the aortas of rabbits fed excessive doses of vitamin D to exhibit dissecting aneurysms, though the medial damage was severe. That hypertension was the factor lacking in these animals is a reasonable conclusion.

In the case reported here no measurements of blood pressure were made. However, the hypertrophy of the heart, manifest both grossly and microscopically, the great narrowing of the lumens of the vessels arising from the aortic arch and the presence of chronic vascular nephritis in one kidney make it probable that hypertension was present in the animal.

The primary factor in this case was a diffuse atherosclerotic process with an atheromatous ulcer, induced by cholesterol. Atherosclerosis is the agency responsible for a small number of dissecting aneurysms in man. Rupture may occur in relation to an atherocheuma (atheromatous abscess, so called) or to an atheromatous ulcer which has resulted from the rupture of an atherocheuma. The two types of genesis of dissecting aneurysm in the rabbit appear to be identical with the mechanisms observed in man.

SUMMARY

A dissecting aneurysm of the aorta is reported arising in an atheromatous ulcer in a cholesterol-fed rabbit which was allowed to live for three years after cholesterol feeding was stopped.

The relation of atherosclerosis in man to experimental atherosclerosis in the rabbit is discussed. The necessity of comparing standard (late) human atherosclerotic lesions with late atherosclerotic lesions in the rabbit is stressed.

The causation of dissecting aneurysm appears to be identical in man and in the experimental rabbit. In both the important causative factors are medionecrosis or atherosclerosis, with hypertension.

BEHAVIOR OF TUBERCLE BACILLI FOLLOWING THEIR INTRAVENOUS INJECTION INTO A RESISTANT ANIMAL (RAT)

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Rats have been shown to be highly resistant to infection with tubercle bacilli; in those cases in which tuberculosis develops, it is usually the Yersin type, characterized by enormous numbers of bacilli, together with proliferation of epithelioid cells, and absence of caseation.

This study was undertaken to determine the fate of tubercle bacilli when they are injected intravenously into an animal, namely, the rat, which is regarded as highly resistant to them. The recent work of Hehre and Freund suggests that so far as the lungs are concerned there is an initial reduction between about the second and tenth weeks, though the rats which died six months or more after injection showed large numbers of the bacilli in their lungs. The figures published by these authors were derived from counts on sections of lung, liver and spleen; each represents the number of organisms seen in 100 microscopic fields. This technic was adopted in the present investigation, and, in addition, the number of colonies which grew from 1 mg. of each of these organs was determined by cultural methods.

Albino rats about 6 months old and weighing approximately 150 Gm. were used. An injection of 1 mg. of living tubercle bacilli suspended in 0.1 cc. of saline solution was made into the femoral vein under ether anesthesia. The Ravenel strain, isolated more than thirty years ago by Dr. Mazyck Ravenel, was used; this strain is of the bovine type and is highly virulent, one intravenous injection of 0.00001 mg. being sufficient to kill a rabbit in three to six months, with extensive tuberculosis. In these respects the experiment was identical with that of Hehre and Freund.[1]

The rats were subsequently killed at various intervals. The chest and abdomen were opened under sterile conditions, and specimens of tissue were removed from the center of the lower lobe of the right lung, the center of the right lobe of the liver and the middle of the spleen. Each of these specimens was then treated as follows:

1. A part was fixed in solution of formaldehyde U. S. P. diluted 1:10, for twenty-four hours, and was stained by the Ziehl-Neelsen method for tubercle bacilli and also with hematoxylin and eosin. An attempt was made to obtain a rough estimate of the volume of tissue which was examined when the number of

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1. Hehre, E., and Freund, J.: Arch. Path. **27**:289, 1939.

organisms in 100 microscopic fields was counted. All sections were cut at 6 microns, and the diameter of the microscopic field was found to be 0.15 mm.; hence the volume of tissue in 100 fields was 0.0106 cu. mm. By multiplying the number of bacilli seen in 100 fields by 100, the approximate number of visible bacilli in 1 cu. mm. was obtained.

2. Two hundred to three hundred milligrams of tissue was weighed to the nearest milligram. It was crushed in a mortar, and fifteenth-normal sodium dihydrogen phosphate (NaH_2PO_4) was added in the proportion of 1 cc. to 100 mg. of tissue. After further crushing, 0.2 cc. of this mixture was diluted ten times with the phosphate solution, and 0.2 cc. of the 1:10 dilution was further diluted ten times. Two test tubes containing Petragnani's medium were inoculated with 0.1 cc. of the 1:10 dilution, i. e., with 1 mg. of tissue, and a further two test tubes with the 1:100 dilution, i. e., with 0.1 mg. of tissue. After incubation

TABLE 1.—*Bovine Tubercle Bacilli (Ravenal Strain)*

Rat	Time After Inoculation	Bacilli per Hundred Fields			Colonies per Milligrams of Tissue		
		Lung	Liver	Spleen	Lung	Liver	Spleen
100	½ hour	120	0	0	800	200	80
101	½ hour	240	10	0	400	200	50
104	1 week	25	125	85	300	4,000	1,500
105	1 week	0	215	120	1,000	3,000	5,000
80	2 weeks	700	650	900	7,000	15,000	10,000
81	2 weeks	0	0	0	70	100	100
106	3 weeks	530	15	20	150	40	400
107	3 weeks	540	295	320	1,000	400	1,000
82	4 weeks	250	150	65	3,000	1,000	1,500
83	4 weeks	225	65	185	1,500	1,500	1,000
84	6 weeks	430	105	45	3,000	400	600
85	6 weeks	370	35	135	5,000	300	300
88	2 months	1,760	75	130	∞(15,000)*	1,500	2,000
89	2 months	2,630	55	315	∞(9,000)	1,000	2,000
90	6 months	345	15	10	4,000	1	200
91	6 months	∞	65	40	∞(15,000)	20	400
92	6 months	105	80	15	∞(10,000)	50	500
93	6 months	∞	0	45	∞(20,000)	75	1,000
94	6 months	180	0	5	∞(12,000)	750	750

* The symbol ∞ is used to indicate that the number of colonies was too large to count.

of the cultures at 37 C. for about three weeks colonies began to appear, and these were counted from time to time until the end of the sixth week. A mean figure was then assessed from the numbers of colonies in the four test tubes and was expressed in terms of the number of colonies growing in one of the tubes containing the 1:10 dilution. It was approximately the number of colonies in 1 mg. of tissue.

Three rats died during the course of the experiment. The results obtained on the remaining 19 rats are shown in table 1.

RESULTS

Lungs.—During the first two months the numbers of bacilli indicated a tendency to multiply, and in all the rats killed two to six months after injection cultures showed the numbers were considerably increased. It was impossible to estimate a mean figure for the number of bacilli in 100 fields in the animals killed at six months because in 2 of the 5 rats the bacilli were too numerous to count. Also, in the rats killed at six months the number of colonies (stated in parenthesis) was assessed from the findings in the 1:100 dilutions, as all the tubes containing the 1:10 dilution showed an infinite number of colonies.

Liver and Spleen.—Table 1 shows that there was an initial multiplication of bacilli in these organs; this increase was not maintained, a much smaller number being found at the end of six months.

The large number of colonies and bacilli found in rat 80 as compared with the other rats killed after the shorter intervals cannot be easily explained. The animal appeared to be healthy, and at autopsy no intercurrent disease was found. It is evidence that occasionally a rat shows little resistance to the multiplication of tubercle bacilli after intravenous inoculation.

In order to investigate more fully the behavior of tubercle bacilli during the first few weeks, a further series of rats was given intravenous injections of a small quantity of tubercle bacilli, namely, 0.01 mg. The Ravenel strain was

TABLE 2.—*Bovine Tubercle Bacilli (Ravenel Strain)*

Rat	Time After Inoculation	Bacilli per Hundred Fields			Colonies per Milligrams of Tissue		
		Lung	Liver	Spleen	Lung	Liver	Spleen
102	½ hour	0	0	0	20	5	15
103	½ hour	0	0	0	40	12	12
96	2 weeks	0	0	1	100	60	500
97	2 weeks	0	0	0	0	300	30
98	4 weeks	0	0	0	0	30	200
99	4 weeks	0	0	0	250	20	150
109	8 weeks	2	0	0	1,000	30	150
110	10 weeks	50	0	0	3,000	60	1,250
111	10 weeks	25	0	0	5,000	80	350

TABLE 3.—*Human Tubercle Bacilli (Jamaica 22)*

Rat	Time After Inoculation	Bacilli per Hundred Fields			Colonies per Milligrams of Tissue		
		Lung	Liver	Spleen	Lung	Liver	Spleen
74	½ hour	0	0	0	50	30	30
75	½ hour	0	0	0	100	15	90
76	2 weeks	0	0	0	0	3	3
77	2 weeks	0	0	0	1	10	120
79	4 weeks	0	0	0	150	15	20
112	8 weeks	0	0	0	200	5	40
113	10 weeks	0	0	0	500	3	7
114	10 weeks	0	0	0	..	0	7

used, as before, and, in addition, 9 rats were given the same quantity of Jamaica 22, a virulent human strain. The results are shown in tables 2 and 3.

Discrepancies are bound to occur when only small parts of organs are examined. In these experiments the specimens were taken from the same part of the organ in every rat, regardless of whether that part appeared to be diseased or not. The results doubtless would have been different if the specimens had been taken from those parts which on gross dissection appeared to have lesions. Errors in the counting of tubercle bacilli are exemplified by the findings in rats 100 and 101 of experiment 1. These animals were both killed half an hour after inoculation. By direct counting the tubercle bacilli in sections of the lungs, rat 101 was found to have more than twice as many bacilli as rat 100, yet the number of colonies grown from rat 100 was twice that from rat 101.

In counting 100 microscopic fields a very small volume of lung was studied, estimated as only 0.0106 cu. mm. Further, in specimens obtained a short time after inoculation the bacilli were for the most part clumped together in the small

blood vessels of the lungs. Later they were seen to have migrated into the pulmonary substance, and by about the third week most of them were lying within epithelioid cells. In the rats killed at six months they were in large numbers in epithelioid and giant cells scattered throughout the lungs. Microscopic examination did not reveal whether the organisms were alive or dead.

With the culture method a much larger volume of tissue was used; a suspension was prepared from 200 to 300 mg., and 1 mg. of this was planted on the culture medium. The liver and spleen could easily be ground in a mortar, but considerable difficulty was experienced with the lungs.

To assist the breaking up of the clumps, the sodium dihydrogen phosphate solution was used, but microscopic films showed that this procedure even after prolonged grinding was by no means always successful. Failure to break up clumps diminished the number of counted colonies. With this technic, the colonies grew well, and not more than a dozen of more than 400 culture tubes became contaminated.

In the experiments shown in tables 2 and 3 the counting method was valueless on account of the scarcity of the bacilli. On the other hand, in table 1, in those cases in which the bacilli were at all plentiful the figure representing the number of organisms per cubic millimeter (obtained by multiplying all the results by 100) was considerably larger than the number of colonies which grew from 1 mg. of tissue. This difference is perhaps explained by the failure to break up clumps in the process of grinding.

Tables 2 and 3 show that tubercle bacilli in small numbers are not demonstrable in the tissues by microscopic examination of sections, whereas cultures prepared from weighed quantities of the tissue show that they are present in considerable abundance.

The results when the cultural method was used clearly indicate that the Ravenel strain had multiplied very considerably in the lungs by the end of the eighth week. In general there was scant multiplication in the liver and the spleen. In some animals there was an increase in the spleen, but the figures for the liver showed little change. With the Jamaica 22 strain there was a tendency to multiply in the lungs. Unfortunately, no reading was possible on rat 114, owing to contamination; the liver and spleen of this animal suggested a slight diminution in numbers.

In experiments in which large (1 mg. in table 1) and small (0.01 mg. in table 2) quantities of bovine tubercle bacilli were injected intravenously into rats, cultures demonstrated that the tubercle bacilli steadily multiplied in the lungs. In the liver and spleen there was much less multiplication; these organs evidently inhibited but did not destroy the bovine tubercle bacilli. A somewhat similar relation was found by Lurie [2] in rabbits inoculated with bovine tubercle bacilli.

Notwithstanding the apparent resistance of the rat to tuberculous infection, tubercle bacilli may multiply with little apparent restraint in the lungs and in some instances in the liver or spleen. In association with scant antibody formation [1] there is multiplication of acid-fast bacilli within epithelioid cells with the production of lesions that recall those of leprosy.

SUMMARY

Tubercle bacilli, both bovine and human, introduced into the blood stream of the rat remain viable in the lungs, liver and spleen.

2. Lurie, M. B.: J. Exper. Med. **55**:31, 1932.

Direct microscopic examination of sections is an unsatisfactory method for determining the number of living tubercle bacilli and fails to demonstrate them when few are present. Counting of colonies in cultures made with weighed quantities of tissue, though subject to error caused by clumping of bacilli, determines more exactly the relative number of micro-organisms in internal organs at intervals after infection.

Bovine tubercle bacilli on arrival in the lungs of rats after intravenous inoculation increase almost continuously so that great numbers are present after from two to six months. Human tubercle bacilli on arrival in the lungs after intravenous injection multiply, but the increase which occurs is much less than that with the bovine type.

Bovine or human tubercle bacilli that reach the liver and spleen after intravenous inoculation persist and in a few instances multiply; multiplication is usually inhibited, however, so that after six months the average number is less than that at an earlier period.

Case Reports

METASTATIC ADENOCARCINOMA IN THE LOWER JAWBONE

RUDOLF KRONFELD, M.D., D.D.S., CHICAGO, AND JOSEPH P. WEINMANN, M.D., NEW YORK

As far as we have been able to ascertain, the histologic aspects of a metastatic tumor in the jawbone have been reported only once. The reason for this is not that such metastases are rare but rather that the jawbones are almost never included in an autopsy or in a microscopic study of postmortem specimens. This scarcity of published material was one of the considerations that prompted the present report. A second was the possible correlation between the recent experimental work with transplantation of the epithelium of the urinary tract and the osteogenic properties of carcinoma of the prostate.

Hofer [1] and Euler [2] reported the changes in bone in the presence of primary malignant tumors of the jaws. Hofer was particularly concerned with the question as to which cells are responsible for the resorption of bone. However, in none of the cases reported by these two authors were there metastases from distant tumors. Skillen,[3] on the other hand, described the changes in the maxilla and mandible of a woman of 40 years with carcinoma of the breast. Most of the jawbone had been invaded by tumor metastases; in places the alveolar bone had been destroyed, and tumor cells lay in contact with the tooth surface. Many good photomicrographs illustrated this. Throughout the jaws, simultaneous resorption of bone and formation of new bone were found. No detailed clinical history of the patient was given, however, and no conclusions were drawn as to the possible significance of the changes observed in the bone.

REPORT OF A CASE

A white man 63 years old was well until about six months before death. At that time he experienced increasing pain in the joints, which made movement difficult and impaired his gait. Pain developed in the chest. He became progressively weaker and lost 45 pounds (20 Kg.) in the last three months. On his admission to the hospital a diagnosis of inoperable carcinoma of the prostate with extensive metastases was made. The patient died of bronchopneumonia.

Autopsy.—The postmortem examination disclosed adenocarcinoma of the prostate gland with metastases to the urinary bladder, rectal mucosa, liver, lungs, spleen, right and left inguinal lymph nodes, periaortic and iliac lymph nodes, a

From the Foundation for Dental Research of the Chicago College of Dental Surgery, Chicago, and the Department of Oral Anatomy, Columbia University Dental School, New York.

1. Hofer, O.: Ztschr. f. Stomatol. **23**:522, 1925.
2. Euler, H.: Deutsche Monatschr. f. Zahnh. **43**:701, 1925.
3. Skillen, W. G.: J. Am. Dent. A. **17**:1678, 1930.

femur, a lumbar vertebra, the sacrum and ribs. There was confluent bronchopneumonia of the left lung. Benign cortical adenoma was present in both adrenals. There were moderate hypertrophy of the heart and syphilitic aortitis. Emaciation was marked.

Gross Specimen.—The specimen consisted of the upper and lower jaws, which had been removed during the autopsy. The teeth were in poor condition; most of the anterior teeth were missing, and, of the posterior teeth, several had been destroyed by caries, so that only the roots were present. The roentgenograms taken before decalcification revealed no significant conditions of bone that could be interpreted as indicating changes due to a tumor metastasis.

Method of Investigation.—The jaws were fixed in solution of formaldehyde U. S. P. and alcohol and decalcified in 5 per cent nitric acid. Then the upper

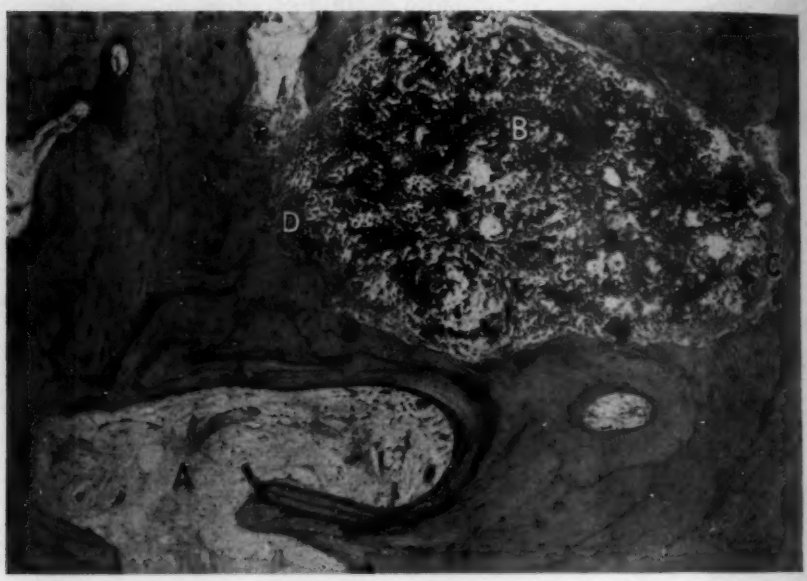

Fig. 1.—Bone and bone marrow in the upper jawbone of a man who died of carcinosis originating from an adenocarcinoma of the prostate. *A*, gelatinous and fibrous bone marrow with a few fat cells; *B*, red bone marrow; *C*, compressed original connective tissue of the fibrous marrow; *D*, osteoclasts and Howship's lacunae. Magnification, 65.

jawbone was divided into eleven parts and the lower jawbone into nine parts, and the parts were embedded in celloidin (a preparation of pyroxylin). The sections were stained with hematoxylin and eosin.

Histologic Observations.—(*a*) Upper Jawbone: The upper jawbone was entirely free of tumor metastasis. The bone marrow consisted of all three types of marrow. Most of the marrow spaces contained adult fat marrow. In addition, there was a considerable amount of fibrous marrow, and scattered through the upper alveolar process were small islands of red cellular marrow. Most of the bone surface was aplastic; only in some areas, where the red marrow seemed to have been on

the increase, was there lacunar resorption of the surrounding bone trabeculae. A typical area of this kind, taken from the upper jawbone, is shown in figure 1. At *A* there is fibrous marrow with occasional fat cells embedded in an edematous, gelatinous-appearing matrix. At *B* is an island of red marrow, probably of erythroblastic nature; it is surrounded by a narrow margin of fibrous tissue, *C*, which appears to be the displaced residue of the original fibrous marrow. On the surface of the surrounding bone, numerous osteoclasts, *D*, are located in Howship's lacunae.

These histologic changes in the upper jawbone may be interpreted as secondary reactions to the emaciating disease of this patient. Fibrous changes in the marrow of the jawbones are known to accompany chronic dental infection of the type present in this jawbone. Gelatinous degeneration of the bone marrow is brought about by the cachexia accompanying malignant disease. Islands of red marrow are almost always found in adult jawbones. However, in this person the progressive character of the change in the red marrow suggests a correlation to the secondary anemia associated with carcinomatosis.

(*b*) Lower Jawbone: The lower jawbone was of particular interest because of the extensive invasion of the metastasizing carcinoma. It was impossible to determine from which side or along what pathway the invasion took place, because the bone was rather uniformly infiltrated throughout the mandible. A general view of an anteroposterior section through the lower left third molar and the root fragments of the second molar is shown in figure 2, upper part. Most of the bone marrow, except for the area at the alveolar crest, is densely infiltrated with tumor masses. The tumor has also invaded the mandibular canal, *MC*, and in some places has come in contact with the tooth surface. Along the mandibular canal, the tumor has grown from the adjacent cancellous bone through multiple resorption channels toward the inner bone surface of the canal (fig. 2, lower part). Here its growth appears to have been temporarily checked by the dense fibrous sheath, *C*, surrounding the mandibular vessels and nerves, so that it spreads along the bone surface and has not yet invaded the nerve-vessel bundle proper. In the vicinity of the tumor there is formation of new bone, *NB*. The border between original and new bone is indicated by a dark cementing line. The nature and significance of the new bone will be discussed in connection with some of the later illustrations.

Figure 3, upper part, shows a field in the compact bone of the outer cortical plate of the mandible in the region of the right first molar. The larger haversian canals contain tumor masses, which follow the general course of the tissue spaces. Along the walls of the invaded haversian canals, new bone is being laid down. This is particularly plain in the large haversian canal, H_2, in which a mass of tumor cells is almost completely encircled by new, partly still uncalcified bone, *B*. On higher magnification of one of the haversian canals, the character and arrangement of the tumor cells are visible (fig. 3, lower part). The tumor consists of columnar cells, which in some areas are arranged definitely in the shape of acini. The surrounding bone is covered with flat osteoblasts and with a layer of newly formed, uncalcified bone matrix (osteoid, *D*).

The osteoplastic character of this carcinoma is particularly evident in areas like the one illustrated in figure 4, upper part, which shows the inside of a larger marrow cavity that is being invaded by the tumor. The original bone surface can be clearly recognized by a cementing line (*A*). Alongside the entire bony wall of the marrow space, abundant formation of new bone has taken place. This bone has been laid down in delicate trabeculae, the surface of which is densely

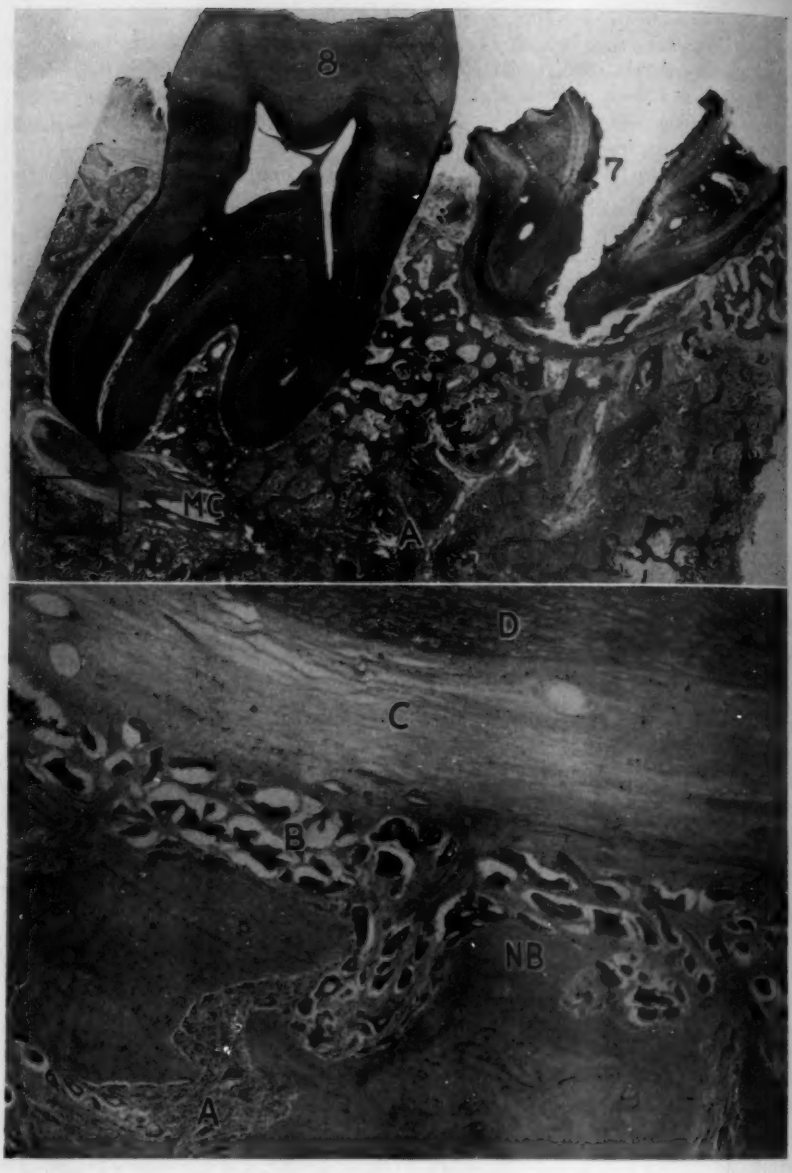

Fig. 2.—Upper: Anteroposterior section through the lower left third molar (*8*) and the root fragment of the second molar (*7*). Note the diffuse carcinomatous infiltration of the bone marrow (*A*) and the invasion of the mandibular canal (*MC*) by tumor cells. The area marked with a square is shown below under higher magnification. Magnification, 4.

Lower: Carcinoma breaking through the bone wall of the mandibular canal. *A*, carcinoma in the fibrous bone marrow; *B*, carcinoma spreading beneath the endosteum lining the mandibular canal; *NB*, new bone in the vicinity of the tumor; *C*, fibrous sheath of mandibular vessels and nerves; *D*, mandibular nerve. Magnification, 36.

beset with osteoblasts. There seems to be a definite tendency on the part of the bone to surround the islands and pegs of tumor cells. This is shown in a high magnification of the newly deposited bone (fig. 4, lower part, which is the area

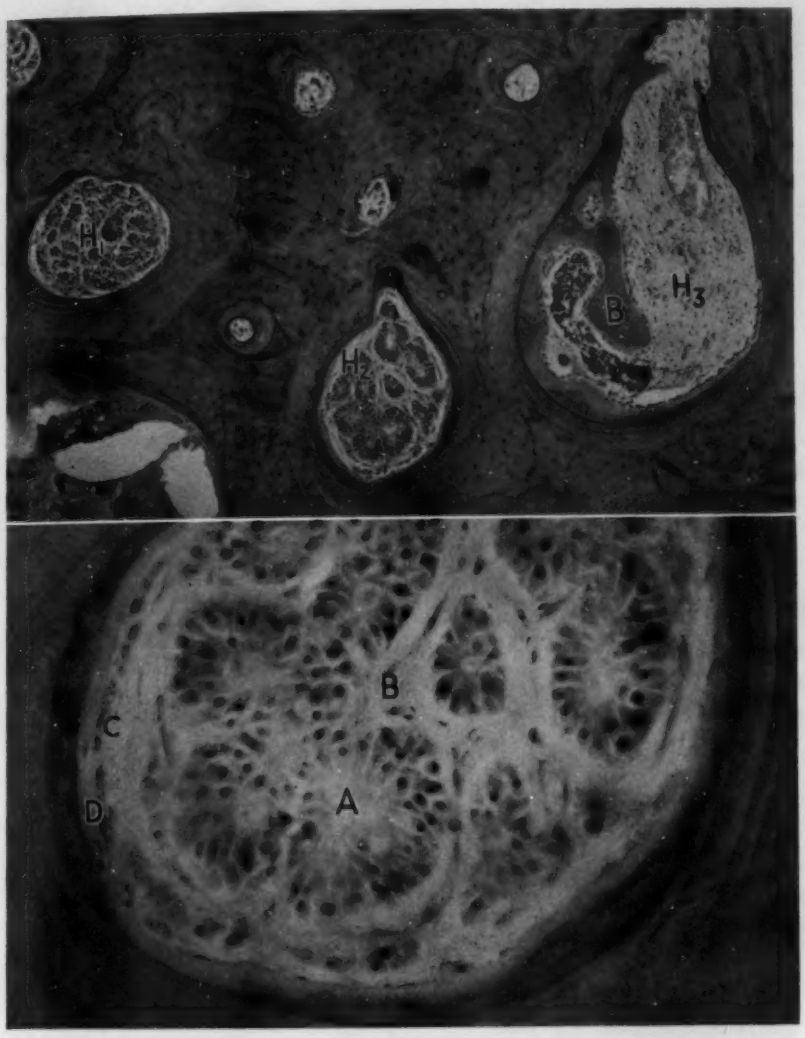

Fig. 3.—Upper: Carcinoma invading the compact bone on the labial plate of the mandible in the first molar region. H_1, H_2, haversian canals containing tumor acini; H_3, a large bone canal containing fibrous marrow and being invaded by tumor cells; *B*, new bone surrounding the tumor cells. Magnification, 65.

Lower: High magnification of haversian canal, H_2, shown in upper part. *A*, acini of the adenocarcinoma; *B*, connective tissue stroma of the tumor; *C*, osteoblasts; *D*, uncalcified new bone matrix. Magnification, 275.

marked by a square in fig. 4, upper part). At *C*, cross sections of small clusters of carcinoma cells are visible. They are surrounded by bone, most of which is still uncalcified. Only in the center of the trabeculae has some calcification taken

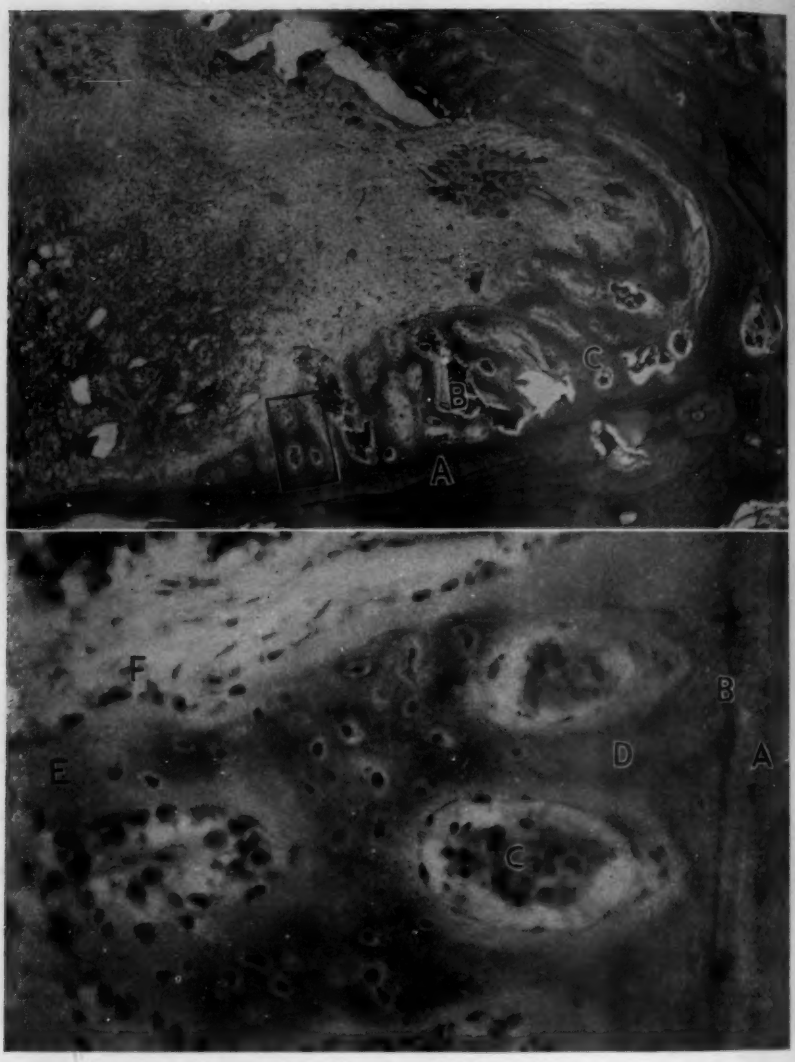

Fig. 4.—Upper: Portion of marrow cavity containing fibrous marrow. *A*, connecting line indicating the original bone surface; *B*, tumor tissue; *C*, new bone trabeculae proliferating from the original bone surface toward the tumor. Magnification, 32. The area marked with a square is shown below under high magnification.

Lower: High magnification of the area marked with a square above. *A*, old bone; *B*, cementing line; *C*, tumor cells; *D*, new calcified bone; *E*, newly formed, still uncalcified bone; *F*, osteoblasts. Magnification, 283.

place, as indicated by the dark blue hematoxylin stain. The bone surface stains only pale pink with eosin. The widest layer of uncalcified bone matrix is at the ends of the trabeculae where the bone has been actively growing toward the tumor cells that have invaded the fibrous bone marrow.

COMMENT

It is generally known that in about 70 per cent of all cases of carcinoma of the prostate the lesion is accompanied by skeletal metastases (Ewing [4]; Geschickter and Copeland [5]; Warren, Harris and Graves [6]). All of these are of osteoplastic character. Although areas of resorption can usually be observed microscopically, the formation of bone far exceeds the destruction of bone. Other types of carcinoma also occasionally produce bone-forming metastases, but carcinoma of the prostate ranks first among those with osteoplastic metastases.

Several explanations have been offered for the osteoplastic reaction of the connective tissue to certain metastatic types of carcinoma. Geschickter and Copeland suggested that the invasive power of the tumor is low enough to allow the proliferating bone to keep pace with the invasion. Thus, they look on this process as a form of protective reaction on the part of the bone. Recklinghausen (cited by Ewing) attributed the formation of bone to a low grade chronic inflammatory reaction of the connective tissue. He spoke of carcinomatous ostitis, caused by obstruction and stasis in the capillaries of the bone marrow, produced by tumor emboli, which resulted in reactive growth of the connective tissue and bone. Axhausen [7] emphasized the possibility, suggested first by Recklinghausen, that the carcinoma cells liberate substances that act as a chemical irritant and thus cause proliferation of bone. This opinion is also given by Goetsch,[8] who uses it as an explanation for the occurrence of bone formation at a considerable distance from the actual tumor tissue.

Recent experimental work of Huggins [9] showed that transplantation of epithelium from the urinary tract into certain susceptible connective tissue areas results in the extraskeletal formation of bone. The transplanted epithelium itself does not become ossified, but bone develops in the connective tissue adjacent to the actively growing epithelial tissue. In view of these observations, it might be suggested that the epithelial cells of the carcinoma of the prostate, being epithelium of the urinary tract, retain the power to stimulate osteogenesis in susceptible connective tissue. There can be no doubt but that the connective tissue of the endosteum and bone marrow is more capable of reacting by the formation of bone than any other connective tissue of the body.

4. Ewing, J.: Neoplastic Diseases, Philadelphia, W. B. Saunders Company, 1934.

5. Geschickter, C. F., and Copeland, M. M.: Tumors of Bone, New York, American Journal of Cancer, 1931.

6. Warren, S.; Harris, P. N., and Graves, R. C.: Arch. Path. **22**:139, 1936.

7. Axhausen, G.: Virchows Arch. f. path. Anat. **195**:358, 1909.

8. Goetsch, W.: Beitr. z. path. Anat. u. z. allg. Path. **39**:218, 1906.

9. Huggins, C. B.: Arch. Surg. **22**:377, 1931.

Recently Ashburn [10] reported the occurrence of bone and cartilage within a primary carcinoma of the prostate. He referred to the work of Huggins as offering a possible explanation of this observation.

SUMMARY

This report deals with the extensive metastasis of an adenocarcinoma of the prostate to the mandible in a man of 63 years. The connective tissue and bone marrow reacted to the invading tumor by extensive formation of new bone. This bone was laid down in the immediate vicinity of the neoplasm, and part of it is still uncalcified.

There is a possible correlation between the osteoplastic reaction to this carcinoma and the experimental observations of Huggins concerning the osteoplastic property of epithelium transplanted from the urinary tract. It is suggested that the cells of this carcinoma retained their power to stimulate formation of bone in susceptible connective tissue.

10. Ashburn, L. L.: Arch. Path. **28**:145, 1939.

PARAFFINOMA OF THE LUNG WITH SECONDARY TUBERCLE-LIKE LESIONS IN THE LIVER AND SPLEEN

HENRY PINKERTON, M.D., AND VICENTE MORAGUES, M.D., ST. LOUIS

Two fairly distinct types of lesion may be found as a result of the entrance of liquid petrolatum into the lungs by way of the trachea.

In 1925 Laughlin [1] called attention to an acute pneumonic process with peculiar histologic features, resulting from the aspiration of liquid petrolatum. This lesion was accompanied by bacterial infection but not by conspicuous fibrosis, the consolidation of the lung tissue being caused largely by the accumulation of oil-laden phagocytes and other inflammatory cells in the alveoli and alveolar walls.

In 1927 Pinkerton [2] reported multiple firm fibrous nodules in the lungs of a 6 year old boy and showed that these tumor-like nodules were composed of large globules of liquid petrolatum, embedded in dense hyaline fibrous tissue. It was pointed out that these nodules were comparable to the so-called paraffinomas produced by subcutaneous injections of liquid petrolatum. The identification of the oil was based on the facts that it did not blacken with osmic acid and that it was insoluble in absolute alcohol.

Although conditions in many respects intermediate between these two types have been described (Pinkerton [2]), it has recently been pointed out by Ikeda [3] that the recorded lesions resulting from the aspiration of oily or fatty substances fall more or less sharply into the two groups described. The latter worker suggested that the early pneumonic process be called "lipoid pneumonia of the infantile type" and the late cicatricial nodular lesion "lipoid pneumonia of the adult type," or paraffinoma of the lung (if liquid petrolatum is the material involved). This classification, though not entirely satisfactory, seems to be of considerable practical value.

Approximately 85 cases of the infantile type of lesion as defined in the foregoing paragraph had been reported up to 1936 (Ikeda [3]). The majority of the patients were infants and children. Pinkerton [2] showed that oily and fatty substances other than liquid petrolatum, notably cod liver oil, olive oil and milk fat, may be responsible for lesions of this general nature, and this has been substantiated by later observations. The latter worker [4] also showed experimentally that the reaction to cod liver oil is characterized by specific histologic features, the most characteristic of which are the shredding of the oil and the intense acid-

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1. Laughlin, A. J.: Am. J. Path. **1**:407, 1925.
2. Pinkerton, H.: Am. J. Dis. Child. **33**:259, 1927.
3. Ikeda, K.: Arch. Path. **23**:470, 1937.
4. Pinkerton, H.: Arch. Path. **5**:380, 1928.

fast staining of the oil by the Ziehl-Neelsen method, a property which it acquires by oxidation in the alveoli. Other somewhat less reliable criteria for identifying other types of oil or fat were described.

The adult type of lesion as defined in a foregoing paragraph (paraffinoma) has proved to be of less frequent occurrence, so far as one may judge by the cases reported, although it should be pointed out that both types are probably often overlooked by pathologists not specifically searching for them. A second case of this type was reported by Fischer-Wassels [5] in 1933, six years after the first instance. Ikeda [3] found that 14 additional cases were reported between 1933 and 1936, and added 5 cases of his own. In only 14 of these 21 cases, however, was the diagnosis confirmed by necropsy, the diagnosis in the other 7 depending on clinical and roentgenologic evidence alone.

In the original case of paraffinoma (Pinkerton [2]) liquid petrolatum was found in relatively small amounts in the reticular tissue of the spleen, where it had obviously been carried by the blood stream. In the case to be described here the left lung contained a single large paraffinoma, although the histologic picture in many other areas was much like that of the infantile type of lipoid pneumonia. The case is of particular interest because of the fact that the liver and spleen were studded with tubercle-like lesions, ranging up to 3.5 mm. in diameter, which were found microscopically to be encapsulated masses of granular and globular material, consisting largely of liquid petrolatum. The significance of this observation will be discussed later.

REPORT OF A CASE

The patient was a 61 year old white man. In this man, at the age of 41, following a febrile illness diagnosed as influenza, typical paralysis agitans developed. It persisted up to the time of death. During the fifteen year period preceding death, precordial and substernal pain were complained of periodically, with progressive dyspnea. Swelling of the ankles was noted during the last year of life. Eight years before death nephrolithiasis was diagnosed, and four years later a stone was removed from the right ureter. During the last five years of life there were frequent attacks of pharyngitis and bronchitis, with the collection of large amounts of mucus in the throat, and increased salivation, not relieved by atropine.

Dec. 8, 1938, the patient had a chill, with pain in the left side of the chest. Auscultation disclosed rales in both lungs, and roentgen examination showed a picture which was interpreted as consolidation of a portion of the lower lobe of the left lung. Sputum culture showed Pneumococcus type VI, and two injections of 20,000 units of type VI serum were given. The red blood cell count was 3,140,000; the white cell count, 12,450, with 70 per cent neutrophils. The urine showed no albumin, sugar, casts or cells. Death occurred December 13.

Unfortunately, information relative to the use of liquid petrolatum was not obtained from the patient, since the nature of the pulmonary condition was not suspected during life. Subsequent questioning of friends and attending physicians brought out the fact that liquid petrolatum had been taken freely by mouth over a period of several years for the relief of severe constipation.

Necropsy.—The body was emaciated, and there was pitting edema of the ankles and on the inner aspects of the thighs. There were fibrous adhesions between

5. Fischer-Wassels, B.: Frankfurt. Ztschr. f. Path. **44**:412, 1933.

several loops of small intestine. The heart was normal in size. The descending branch of the left coronary artery was markedly calcified but not occluded, and the aorta showed advanced atheromatous changes throughout its entire length.

A layer of shaggy, yellowish white fibrinous exudate covered the lateral and diaphragmatic visceral pleura of the lower lobe of the left lung. In the central portion of this lobe a hard mass was palpated. On section, the mass was seen to be sharply circumscribed, irregularly nodular and roughly spherical in shape. It was 4 cm. in the greatest diameter. Its central third was grayish black, from carbon pigment, and almost woody in consistency, while peripherally the tissue was grayish white and somewhat less firm. The cut surface of the mass bulged slightly above the surrounding lung tissue. Bands of scar tissue, radiating outward from the dense central core, divided the peripheral portion of the mass into several distinct secondary nodules, averaging about 1 cm. in diameter. In appearance and consistency this nodular mass was suggestive of primary or metastatic carcinoma.

Medial to the large fibrous mass was an area of firm grayish consolidation, interpreted as confluent bronchopneumonia. This area represented about one fifth of the entire volume of the lower lobe of the left lung and extended downward to the diaphragmatic surface. Laterally and somewhat anteriorly there was an irregular abscess cavity, 3 cm. in the greatest dimension, lined with fibrinous exudate and surrounded by a heavy fibrous wall 2 to 3 mm. in thickness. This abscess was at a slightly lower level than the fibrous mass and separated from it by a wall of partially crepitant lung tissue about 1 cm. in thickness. From the abscess a sinus tract led to the pleural surface, which, as mentioned earlier, was covered with fibrinous exudate. The relative positions of the paraffinoma, the area of pneumonia and the abscess are seen in figure 1. There were several small areas of consolidation in the upper portion of the lower lobe of the left lung and also in the lower lobe of the right lung.

The liver was moderately enlarged. The external and cut surfaces were studded with firm, grayish white nodules resembling miliary tubercles. These varied in diameter from 0.5 to 3.5 mm., averaging 1.5 mm. In many areas three or four of these lesions were seen per square centimeter of cut surface.

The spleen was about one and a half times the normal size. The pulp was soft and studded with tubercle-like lesions similar to those described in the liver.

The pelvis of the right kidney contained a large brown concretion, irregular in shape and with prolongations into the calices. Nodules like those in the liver and spleen were not observed in either kidney at the time of the necropsy. Unfortunately the kidneys were not saved for more careful examination later on.

Microscopic Examination.—Sections of the hard nodule in the lower lobe of the left lung showed the typical picture of paraffinoma (fig. 2 *A* and *B*). This will not be described in detail, as it was entirely similar to instances previously reported by Pinkerton,[2] Ikeda [3] and others. Sections from the center of the nodule (fig. 2 *A*) showed oil globules embedded in a dense hyaline keloid-like collagenous material. Peripherally, the connective tissue was less dense and contained many inflammatory cells, with frequent giant cells surrounding oil droplets (fig. 2 *B*). The area of pneumonia and the abscess showed the characteristic microscopic pictures of these conditions, but oil-laden phagocytes were numerous throughout. Sections of smaller nodules from the upper part of the lower lobe of the left lung showed thickened alveolar walls lined with cuboidal epithelium, and oil-laden phagocytes were present in the alveoli and in the alveolar walls. To this, a picture of acute suppurative pneumonia was added in many areas. Sections from

the nodules in the lower lobe of the right lung were not labeled specifically, and as the right lung was not saved, no statement can be made regarding the nature of these lesions.

The oil droplets in the lung tissue did not stain at all with osmic acid, and it may be said here that oil collected from the surface of the solution of formaldehyde in which the lung tissue was kept was found to be insoluble in absolute alcohol and completely resistant to saponification.

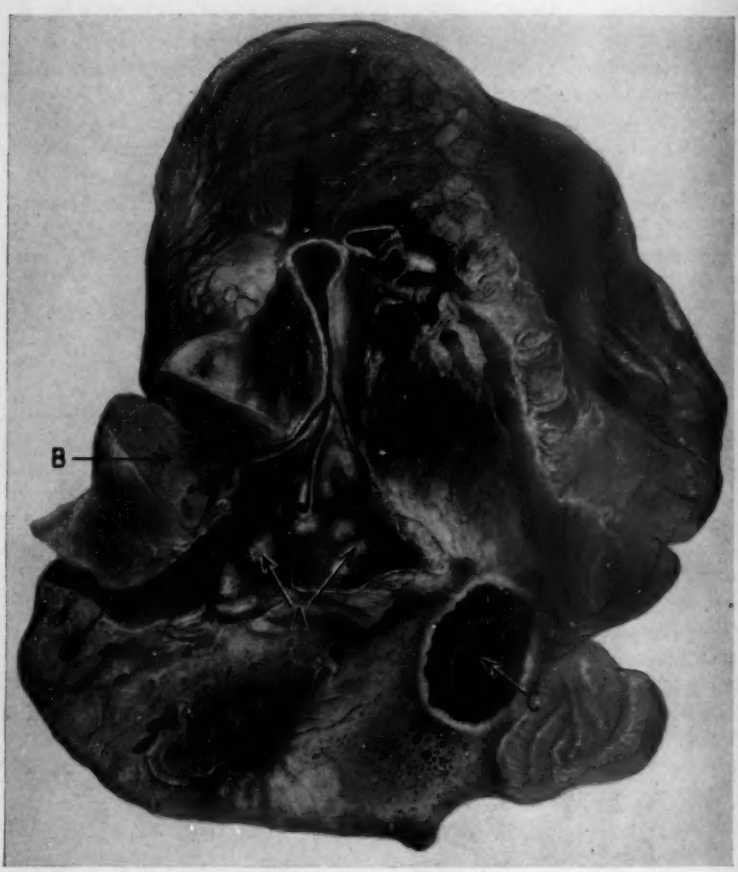

Fig. 1.—Drawing of the left lung, showing (*A*) the paraffinoma in the central portion of the lower lobe, (*B*) an area of pneumonic consolidation medially and at a slightly higher level and (*C*) an abscess cavity laterally and at a slightly lower level.

Peculiar vascular lesions, illustrated in figure 3 *A*, were found in the arteries and veins in several sections of lung tissue. The lumens of these vessels contained globules of liquid petrolatum (as shown by the staining reactions) surrounded by fibrous tissue in such a way as to resemble normal adipose tissue. The picture was like that of organized mural thrombi, the material organized being oil rather

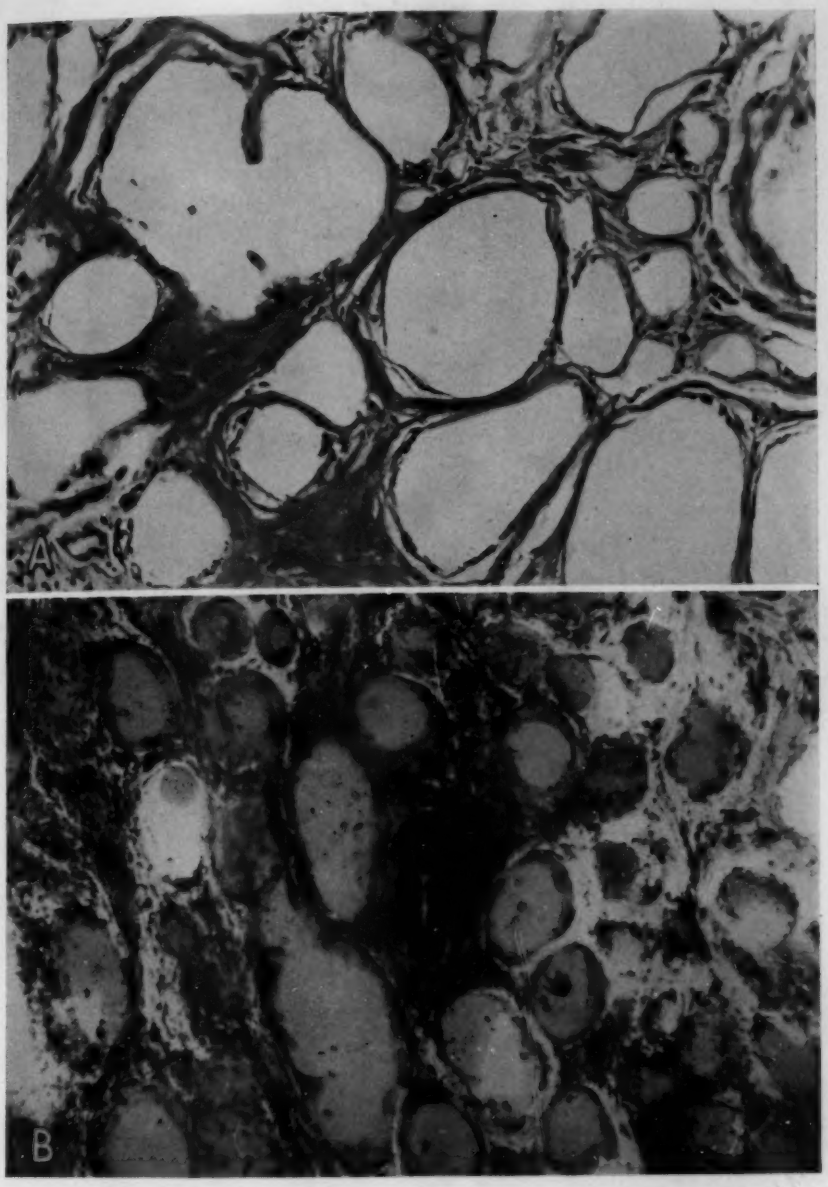

Fig. 2.—*A*, photomicrograph from a paraffin section of the lung, showing the histologic appearance of the dense central portion of the paraffinoma. Spherical masses of oil, represented here by empty spaces, are embedded in a dense hyaline connective tissue. Hematoxylin and eosin stain; × 100. *B*, photomicrograph from a frozen section of the lung, representing the peripheral portion of the paraffinoma. The spherical masses of oil have been stained with scarlet red; × 70.

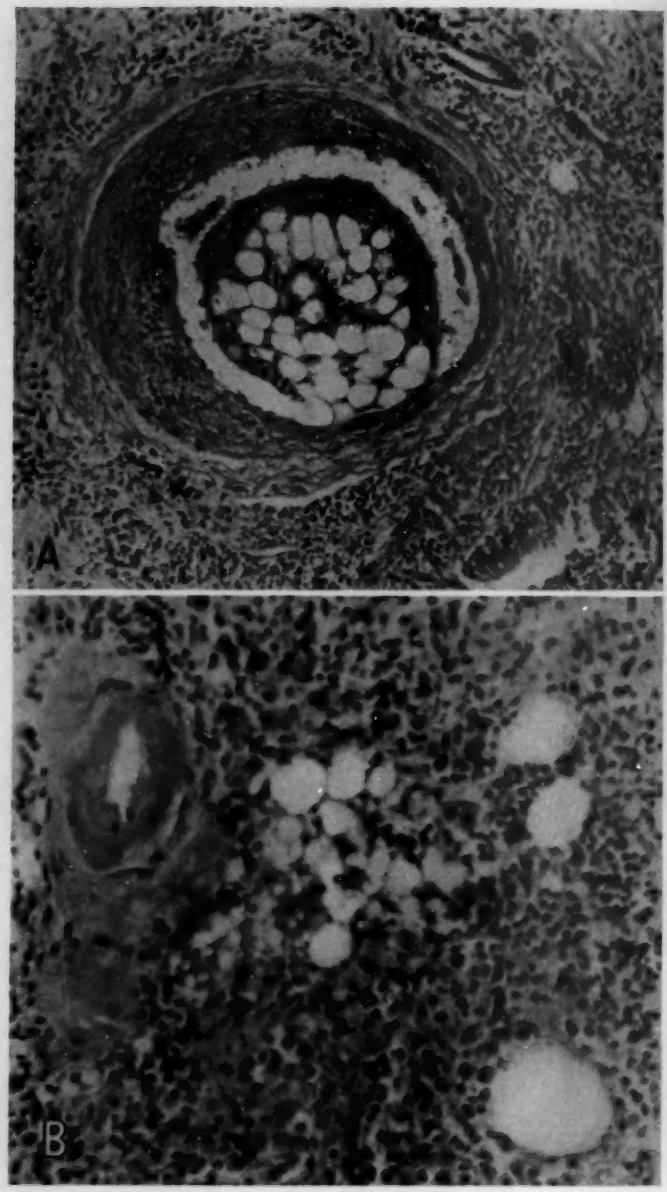

Fig. 3.—*A*, thrombus-like lesion in a pulmonary arteriole, apparently resulting from organization of oil within the lumen of the vessel. Hematoxylin and eosin stain; × 100. *B*, vacuoles representing droplets of liquid petrolatum in the reticular tissue of the spleen. Hematoxylin and eosin stain; × 200.

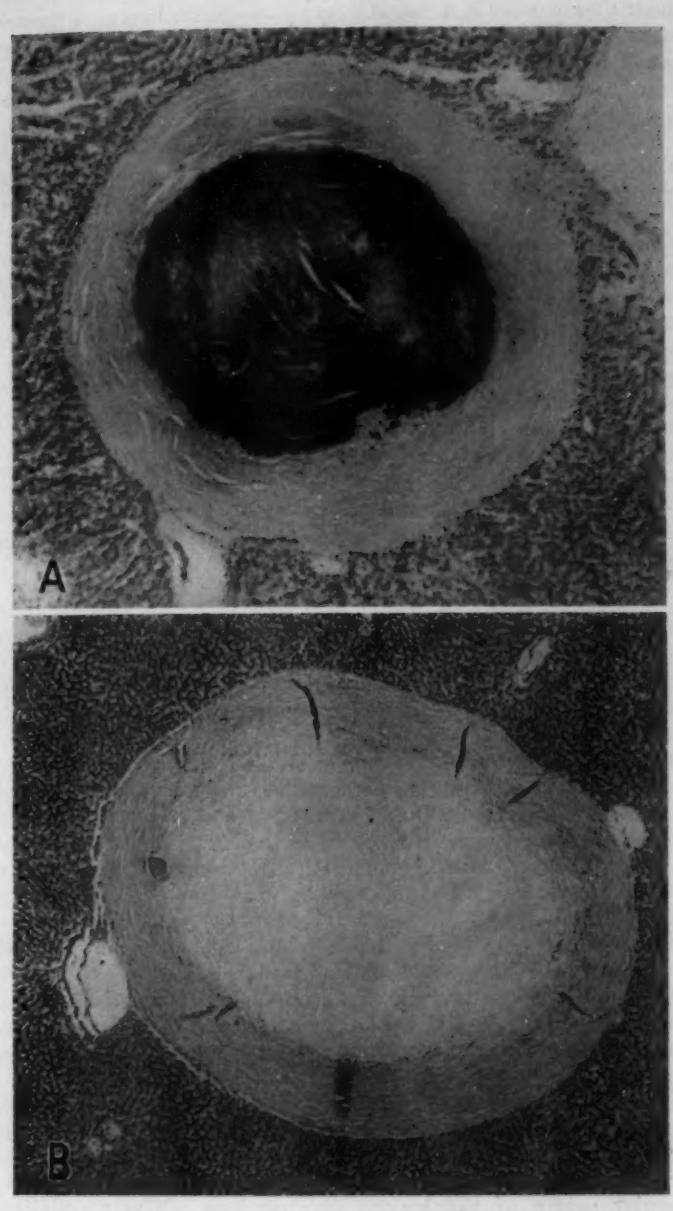

Fig. 4.—*A*, tubercle-like lesion of the liver, with calcium and cholesterol crystals in the central core. Hematoxylin and eosin stain; × 100. *B*, large tubercle-like lesion with hyaline center in the liver. Hematoxylin and eosin stain; × 50.

than fibrin. The mechanism of the production of these lesions is not clear, and it is difficult to understand how the oil gained access to the lumens of these vessels, but it seems likely that these lesions may have been the starting point for oil emboli, which could have caused the peculiar nodular lesions in the liver and spleen. It is clear that these pulmonary lesions are not artefacts, since the organizing connective tissue was always continuous with that in the walls of the vessels.

The lesions in the liver and spleen were apparently identical, and scarlet red stains of frozen sections from the deeply pigmented bronchial lymph nodes revealed a number of similar lesions in these organs. The central portion of each of these nodules was composed of an amorphous mass of globular and granular material staining deep red with scarlet red but not blackening with osmic acid. Peripherally, each nodule was encapsulated by a wide zone of dense fibrous tissue. Many of the nodules contained precipitated calcium and cholesterol crystals, as well as finely emulsified oil (fig. 4*A*). In addition to these encapsulated masses of oil, both the liver and the spleen contained large amounts of oil in the form of large globules and small droplets, sometimes intracellular and sometimes extracellular, often with surrounding giant cells (fig. 3*B*). None of the oil droplets in the spleen stained with osmic acid. In the liver there was some infiltrated fat, which could easily be distinguished from the oil by the fact that it stained with osmic acid.

No evidence of oil deposition was found in four sections of kidney tissue. The kidneys showed focal areas of healed pyelonephritis but no evidence of glomerulonephritis or nephrosclerosis. Brain, pancreas, heart and adrenal tissue were also apparently free from oil deposits.

COMMENT

In this case the neurologic condition is undoubtedly of importance in explaining the aspiration of the oil. In several of the reported cases, however, the paraffinoma has occurred in a person without neurologic damage or other obvious factor predisposing to the entrance of oil into the trachea.

It seems clear beyond a reasonable doubt that the abscess, empyema and focus of bacterial pneumonia were all secondary to the paraffinoma, since all three occurred in close proximity to it. The constriction of bronchioles and blood vessels involved in the area of scar tissue may be regarded as a factor favoring bacterial infection. In this respect, the oil, with the associated fibrosis, may be considered as acting like any other foreign body.

The tubercle-like lesions in the liver and spleen, which were shown to be composed of encapsulated liquid petrolatum, are of particular interest. Such lesions have not previously been described. It seems probable that they originated in blood vessels, although this could not be definitely demonstrated. Their microscopic appearance was not suggestive of tuberculosis.

Young and co-workers [6] recently reported a pulmonary oil tumor in which severe renal damage is believed to have resulted from the metastatic deposition of oil in the glomeruli and blood vessels of the kidney. The aspirated material was believed to be cod liver oil, and the vascular origin of the lesions was obvious. The possibility that serious damage may be done to various organs by liquid petrolatum,

6. Young, A. M.; Applebaum, H., and Wasserman, P. B.: J. A. M. A. **113**:2406, 1939.

cod liver or other types of oil gaining entrance to the circulating blood is worthy of further consideration, especially in view of the probability that such foreign substances may be absorbed from the intestine to some extent without chemical alteration.

SUMMARY

A case of paraffinoma of the lung with terminal abscess formation, bronchopneumonia and empyema is described pathologically. The abscess was apparently secondary to constriction of a bronchiole involved in the cicatricial tissue of the paraffinoma. Organized masses of liquid petrolatum, resembling mural thrombi, were found within the pulmonary vessels. The liver and spleen were studded with tubercle-like lesions, 0.5 to 3.5 mm. in diameter, which were found microscopically to be encapsulated masses of liquid petrolatum, with the addition, in some instances, of calcium and cholesterol crystals. These peculiar lesions in the liver and spleen were undoubtedly secondary to the paraffinoma and probably embolic.

TOPHUS OF THE MITRAL VALVE IN GOUT

JOSEPH J. BUNIM, M.D., AND CURRIER McEWEN, M.D., NEW YORK

While urate crystals may be deposited in numerous structures of the body in the course of chronic gout, they are rarely found in the endocardium. Contrary to the impression given by modern texts, authentic reports of endocardial or valvular tophi are few indeed. In some cases, traces of uric acid, enough to yield a positive reaction in the murexide test, simply impregnate an old calcareous deposit. For example, Coupland,[1] in 1873, reported before the London Pathological Society that at autopsy on a patient who had had gout for eighteen years he found that "the aortic valves were the seat of a deposit strikingly like the gouty deposits occurring in the neighborhood of joints and not at all resembling calcified vegetations." But on submitting the specimen for chemical analysis he received the report that "an indication of the presence of uric acid is obtainable by murexide test, but the bulk of the concretion is composed, as usual, of phosphate and carbonate of lime." Indeed, Lancereaux[2] reported a case of interstitial nephritis terminating in uremia, and though the patient showed no evidence of gout, uric acid was deposited on the mitral valve. Moore[3] published the complete autopsy protocols of 80 patients with chronic gout observed in St. Bartholomew Hospital, and in not a single instance was an endocardial tophus mentioned. Garrod[4] wrote, "I have carefully examined the deposits found on the valves of the heart and the atheroma from the aorta of several gouty patients who had extensive chalk-stones in different parts of the body, but have failed to discover the least trace of uric acid." He quoted Landerer, who found in a patient a white deposit on the inner surface of the aorta, said to consist chiefly of calcium phosphate and calcium carbonate with about 14 per cent uric acid, and Lobstein, who found in a patient a deposit on the mitral valve which had a similar composition. Duckworth,[5] in his treatise on gout, wrote that he knew of no authentic case of gouty endocarditis.

The case which we have observed is therefore of considerable interest.

REPORT OF A CASE

A white man of 63 years, a painter, was admitted to the Third (New York University) Medical Division of Bellevue Hospital on February 13, in a stuporous

From the Third (New York University) Medical Division and the Pathological Laboratories of Bellevue Hospital and the Department of Medicine of New York University College of Medicine.

1. Coupland, S.: Tr. Path. Soc. London **24**:69, 1873.

2. Lancereaux, E.: Gaz. méd. de Paris **23**:187, 1868.

3. Moore, N.: St. Bartholomew's Hosp. Rep. **23**:289, 1887.

4. Garrod, A. B.: Treatise on Gout and Rheumatic Gout (Rheumatoid Arthritis), ed. 3, London, Longmans, Green & Co., 1876, p. 204.

5. Duckworth, D.: A Treatise on Gout, Philadelphia, Blakiston, Son & Co., 1889.

state, unable to give a detailed history. For the past twenty years he had noted large nodules over the elbows and knees with deformity and stiffness of the small joints of the hands, wrists and knees. During the past six years cheesy material escaped from ulcerated surfaces over both tibias. The family, past medical and dietary histories were unobtainable.

On admission he appeared stocky and well nourished but apathetic and seriously ill. He was only slightly dyspneic, not orthopneic or cyanotic. Large tophi, several centimeters in diameter, were situated symmetrically just distal to the olecranon and patella. The olecranon and prepatellar bursae, as well as those over the dorsal aspects of both wrists, were distended, irregular and cystic, although not tender. The hands were clawlike and showed interosseous wasting, ulnar deviation of the fingers, fusiform swelling of metacarpophalangeal and of several proximal interphalangeal joints. The extensor tendon sheaths of several fingers were distended and fluctuant. Numerous tophi were distributed over both hands near the digital articulations. Paronychial suppuration of one finger and one toe was noted (fig. 1 *A*). There were no tophi in the auricular cartilages.

On the anterior surface of the middle third of each tibia appeared an irregular deep ulcerated area, about 5 cm. in diameter, discharging blood and white cheesy material (fig. 1 *B*). The latter was soft, not gritty, and a murexide test was positive. Microscopically, it consisted of long needle-like urate crystals.

The eyegrounds showed sclerotic vessels with arteriovenous compression and irregular areas of retinal degeneration. The cardiac apical impulse was not palpable; the rhythm was regular and the rate 100 per minute. No gallop or murmurs were noted. There were no signs of heart failure. The blood pressure was 200 systolic and 98 diastolic. An electrocardiogram presented a low, diphasic T in lead I and sinus tachycardia, with a rate of 110 per minute. The rest of the physical examination gave negative results. The temperature was irregular, fluctuating between 99 and 103 F.

The specific gravity of the urine varied from 1.010 to 1.015, a trace of albumin was consistently present, and there were 10 to 15 red blood cells and 5 to 10 white blood cells per high power field, and also finely granular casts. The blood showed 3,150,000 red cells, 14,000 white cells, with a normal differential formula, and 70 per cent hemoglobin. The blood nonprotein nitrogen varied from 112 to 150 mg. and uric acid from 7.3 to 9.0 mg. per hundred cubic centimeters (Folin method); the blood creatinine level was 2.5 mg. per hundred cubic centimeters. The roentgen findings were characteristic of gout.

The patient followed a progressively downward course, becoming irrational, delirious, incontinent and finally comatose. He died thirteen days after admission. The clinical diagnosis was gout and chronic diffuse glomerular nephritis.

Autopsy.—Autopsy was performed within twenty-four hours after death. The external appearance conformed to the clinical description given.

The heart weighed 570 Gm. and showed preponderant left ventricular hypertrophy. Close to the free border of the posterior mitral leaflet appeared a sharply circumscribed concretion, 4 cm. long and about 0.5 cm. thick. Material removed from this concretion consisted of long needle-like crystals resembling those of sodium urate. There were no endocardial ulcerations or vegetations. Within the anterior mitral leaflet a few yellowish atheromatous plaques were seen. The tricuspid, pulmonary and aortic valves were normal. The coronary arteries showed diffuse sclerosis with mild narrowing. The aorta showed moderate atherosclerotic changes.

The kidneys weighed 150 Gm. each and were pale gray. The capsule was adherent and could not be stripped without adherent parenchyma. The surface was made up of fine and coarse nodules with an occasional large cyst.

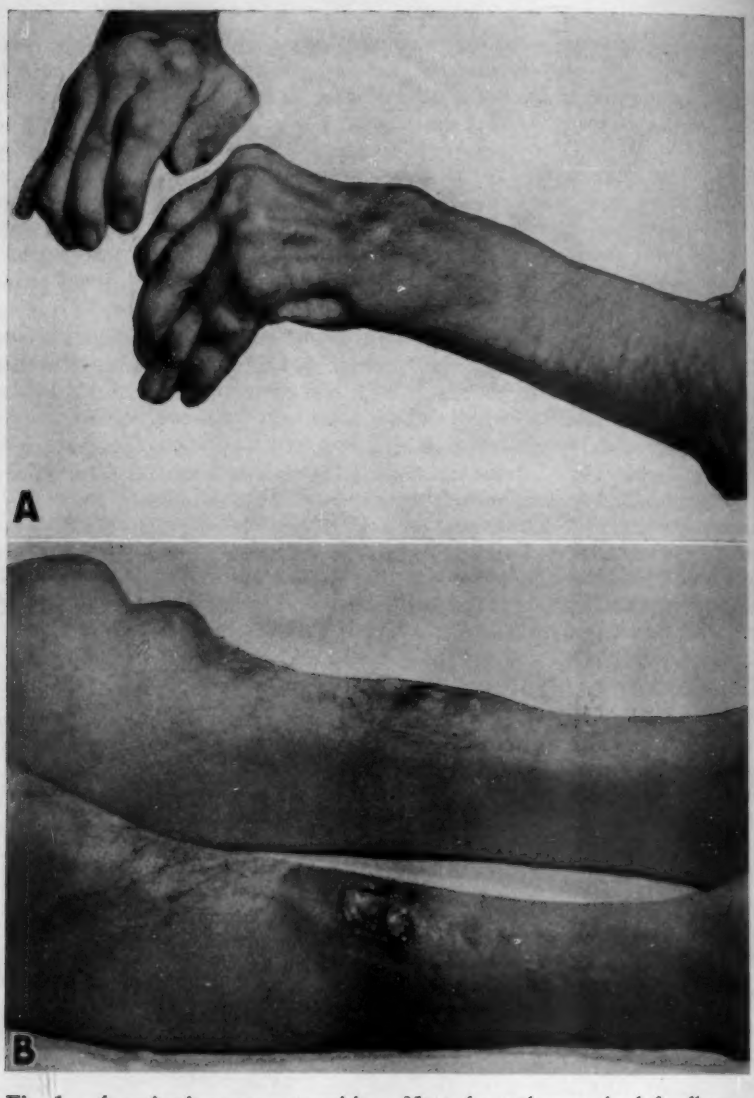

Fig. 1.—*A*, patient's upper extremities. Note the tophus at the left elbow. *B*, patient's lower extremities. Note prepatellar bursae and ulcers discharging uric acid crystals.

On cut sections no sharp demarcation between cortex and medulla was present. Numerous cysts 0.5 cm. in diameter were noted. There were several pinhead and

slightly larger grayish white foci in the medullary rays. The remaining structures were normal on gross examination.

Microscopic Observations.—Sections were taken through the posterior mitral leaflet. These were fixed in paraffin without decalcification and stained with hematoxylin and eosin. The posterior leaflet showed slight fibrous thickening of the proximal one-third and bulbous enlargement of the distal two-thirds. In the latter were large circumscribed masses of amorphous eosinophilic material, surrounded by fibrous tissue, in which were many multinucleated giant cells of the foreign body type, histiocytes, a few lymphocytes, plasma cells and fibroblasts. There were capillaries and thick-walled vessels in the distal part of the leaflet surrounding the concretions. There was no vascularization of the proximal portion of the leaflet. The overlying endocardium was intact. The annulus showed slight fibrous thickening with radiating interfascicular extensions. The endocardium of the posterior left auricular wall appeared normal.

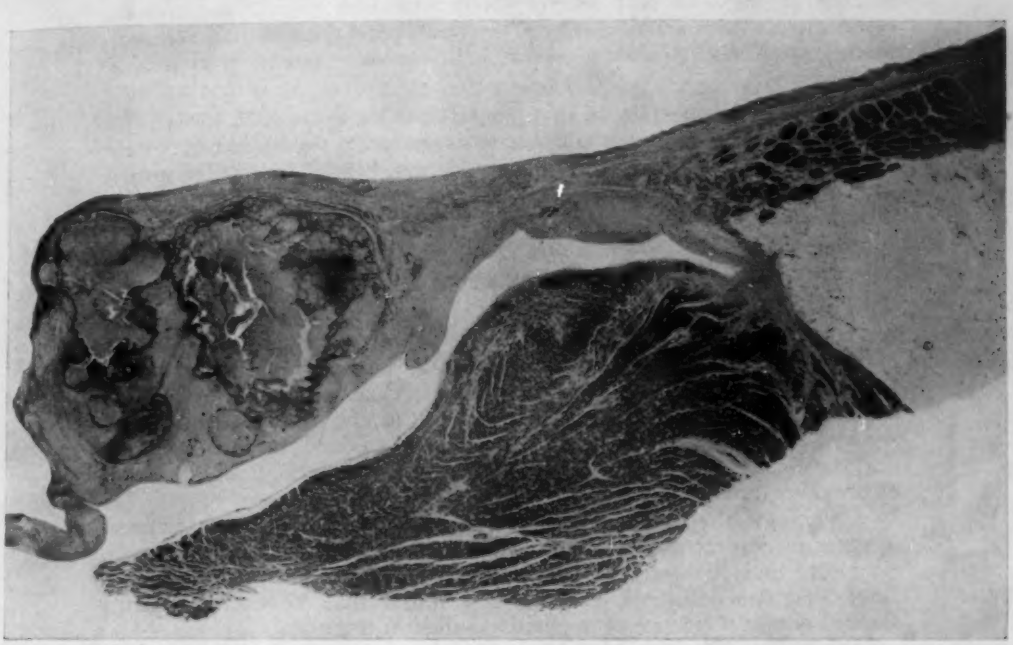

Fig. 2.—Gouty tophus embedded in posterior mitral leaflet; × 12.

Sections through the anterior mitral leaflet showed slight fibrous thickening of the senescent type, without vascularization or inflammation.

In the myocardium there was slight interfascicular fibrosis radiating from some of the medium-sized branches of the coronary arteries. The smaller coronary arteries showed definite intimal thickening.

The aorta showed marked intimal thickening and large atheromas. The arterioles of the spleen and pancreas presented marked intimal hyaline thickening.

Sections through the kidney showed diffuse alterations. The glomeruli were only slightly reduced in number, but few normal glomeruli were present. The majority showed slight thickening of the basement membrane with simplification

of the tuft. A smaller number showed varying degrees of hyalinization of tuft and Bowman's capsule. The interstitial tissue showed a slight diffuse increase with focal lymphocytic infiltration. The afferent glomerular arterioles presented pronounced subendothelial hyaline thickening, and the interlobular and arcuate arteries exhibited marked intimal hyperplasia with diminution of the lumens of the vessels. There were occasional small adenomas in the cortex, and in the medulla were several miliary and larger foci of amorphous eosinophilic material, surrounded by foreign body giant cells.

Pathologic Diagnosis.—The final diagnosis was: numerous subcutaneous tophi; a urate concretion in the posterior mitral leaflet; coronary arteriosclerosis; hypertrophy and dilatation of the cardiac chambers; atherosclerosis and diffuse arteriolosclerosis of the aorta and visceral vessels; advanced arterial and arteriolar nephrosclerosis with urate deposits in the renal medulla and cortical adenomas; lobular pneumonia, pulmonary edema and pulmonary arterial thrombosis or embolism.

Chemical Analysis.—(Dr. Isidor Greenwald and Dr. Solomon H. Rubin). The material removed from the mitral tophus was submitted to the Benedict and Franke test for uric acid, as well as to the murexide test, both of which were strongly positive. The sample also contained cholesterol. No calcium or phosphates were present. The material was insufficient for quantitative studies.

COMMENT

There seems to be enough evidence to warrant the conclusion that the concretion encountered in the posterior mitral leaflet was a tophus of uric acid crystals. This conclusion is based on the microscopic appearance of the crystals, the absence of any basophil material in undecalcified sections of the mitral valve and the chemical analysis, which showed presence of uric acid and absence of calcium. It is noteworthy that the concretion was situated in a valve showing little, if any, evidence of previous inflammatory changes.

Pathologic alterations of the type noted in the kidney are not unusual in cases of chronic gout. They have been described by many observers. Schnitker and Richter [6] reviewed 55 cases of gout studied at the Peter Bent Brigham Hospital, Boston, and found that 17 of the patients showed clinical evidence of nephritis; 5 died in uremia.

While the diagnosis of gout in this case offered no difficulty, it is interesting to note that many of the clinical signs resembled those frequently seen in rheumatoid arthritis.[7]

SUMMARY

A case of chronic gout with a uric acid tophus deposited in the mitral valve is reported. No clinical signs of this unusual lesion were present.

6. Schnitker, M. A., and Richter, A. B.: Am. J. M. Sc. **192**:241, 1936.

7. Ludwig, A. O.; Bennett, G. A., and Bauer, W.: Ann. Int. Med. **11**:1248, 1938.

Laboratory Methods and Technical Notes

BIEBRICH SCARLET-PICRO-ANILINE BLUE: A NEW DIFFERENTIAL CONNECTIVE TISSUE AND MUSCLE STAIN

R. D. Lillie, M. D., Washington, D. C.
Surgeon, United States Public Health Service

In the course of current studies on the staining of connective tissue, a very successful combination, based on the technics of Van Gieson, Mallory and Masson, was encountered. This technic, which may be carried through in less than twenty minutes on paraffin sections of routine material fixed in solution of formaldehyde, demonstrates basement membranes in renal glomeruli and intestinal mucosa and also much of the reticulum of lymphadenoid structures, and stains brilliantly the usual connective tissues in sharp contrast to muscle and cytoplasm.

DESCRIPTION OF TECHNIC

Paraffin sections of material fixed in solution of formaldehyde, U. S. P. (1 part to 4 parts or 1 part to 9 parts water), Orth's fluid or Zenker's fluid are deparaffinized and brought to 80 per cent alcohol as usual. The material fixed in Zenker's fluid is treated with iodine and then with thiosulfate as usual.

1 Stain five minutes in Weigert's acid–iron chloride–hematoxylin or other similar iron-hematoxylin stain.

2. Wash in water.

3. Stain four minutes in a solution made as follows: Biebrich scarlet, 0.2 Gm.; glacial acetic acid, 1 cc., and distilled water, 100 cc.

4. Rinse in water.

5. Stain four to five minutes in a fluid made as follows: aniline blue W. S., 0.1 cc., and saturated aqueous picric acid, 100 cc.

6. Wash three minutes in 1 per cent acetic acid in water.

Dehydrate, clear and mount. In this laboratory an acetone, acetone-xylene, xylene, salicylic acid balsam series is used.

Red corpuscles are orange scarlet, muscle deep salmon pink, cytoplasm gray to pink, nuclei black, basement membranes, reticulum and finely fibrillar connective tissue deep blue, and coarse and hyalinized connective tissue basically blue but perhaps showing red-violet areas.

From the Division of Pathology, National Institute of Health.

Notes and News

University News, Promotions, Resignations, Appointments, Deaths, Etc.—Lauren V. Ackerman, of the University of California Medical School, has been appointed pathologist to the Ellis Fischel State Cancer Hospital, Columbia, Mo.

The Committee on Scientific Research of the American Medical Association has awarded a grant to L. J. Meduna, F. J. Gerty and V. G. Urse, Loyola University, Chicago, for a study of the biochemical changes in patients with schizophrenia under treatment with metrazol, which was originated by Dr. Meduna.

William H. Taliaferro, of the University of Chicago; Karl F. Meyer, of the University of California; James B. Murphy, of the Rockefeller Institute for Medical Research; Stephen W. Ranson, of Northwestern University; R. E. Shope, of the Rockefeller Institute, Princeton; George W. Corner, of the University of Rochester, and Carl F. Cori, of Washington University, St. Louis, have been elected to membership in the National Academy of Sciences.

Calvin B. Coulter, associate professor of pathology in the Long Island College of Medicine, Brooklyn, and bacteriologist in chief at Kings County Hospital, has died at the age of 52 years.

Award.—W. H. Sebrell, surgeon, United States Public Health Service, shares in the Mead Johnson & Company award for "the most outstanding work on vitamin B complex in North America in 1939."

Fellowships. — The Finney-Howell Research Foundation, Baltimore, has renewed seven fellowships for research on the nature and treatment of cancer and has awarded seven new fellowships for the same purpose. The closing date for filing applications for awards for 1941 is January 1 next.

Society News.—The sixth International Congress for Experimental Cytology, which was to have been held in Stockholm, Sweden, from July 25 to August 1, has been postponed indefinitely.

The Illinois Society of Pathologists was organized on April 6, 1940, with J. J. Moore as president, O. T. Schultz as vice president and I. Davidsohn as secretary-treasurer.

Abstracts from Current Literature

To Save Space the Original Titles of Abstracted Articles Sometimes Are Shortened

Pathologic Anatomy

Traumatic Autotransplantation of Splenic Tissue. S. Jarcho and D. H. Anderson, Am. J. Path. **15**:527, 1939.

Two cases are presented in which a person who had undergone splenectomy for splenic rupture was subsequently found to have numerous nodules of spleenlike tissue scattered throughout the peritoneal cavity. Mention is made of 8 additional cases gathered from various sources and of 4 cases in which no definite history of trauma was available. In 2 of these the spleen and the left kidney were shrunken, scarred and distorted. Several analogous cases of a similar condition in an animal are cited from the literature. The experimental evidence is summarized, and it is shown that splenic tissue is susceptible to autoplastic transplantation. The "splenoid" theroy is described and is shown to be a gratuitous assumption, inadequately supported by evidence. It is concluded that the aforementioned nodules found in the peritoneum and omentum of persons who had undergone splenectomy for traumatic rupture of the spleen are due to autoplastic transplantation of particles of spleen torn loose from the main body of splenic tissue and disseminated in part by the aid of hemorrhage.

From Authors' Summary.

Intracranial Aneurysms. C. A. McDonald and M. Korb, Arch. Neurol. & Psychiat. **42**:298, 1939.

McDonald and Korb tabulated 1,125 cases of, verified arterial aneurysm at the base of the brain and added 2 of their own. The earliest recorded cases date back to the middle of the eighteenth century; the latest, to 1937. Four hundred and seven contributions have been perused and arranged chronologically according to the year of publication. In 48 per cent of the cases the aneurysm was located at the internal carotid artery or at the middle cerebral artery; in 15 per cent, at the anterior communicating artery, and in 28 per cent, posterior to the internal carotid artery. The youngest patient was aged 1½ years; the oldest, 87. The pathologic changes present in 67.3 per cent were in the form of sclerosis of the arteries, mycotic emboli and syphilitic changes.

George B. Hassin.

Squamous Metaplasia, Simulating Carcinoma, Associated with Prostatic Infarction. O. S. Culp, Bull. Johns Hopkins Hosp. **65**:239, 1939.

Only 1 previous case of metaplasia in the prostate associated with infarction has been found recorded in the literature. Eight patients, presenting a total of 11 typical infarcts of the prostate, were studied, and in each one squamous epithelium was noted along the periphery of the infarct. In 5 of these patients the cellular changes had been interpreted previously as early carcinoma. The squamous cells in all were confined to the margins of the infarct, and the remainder of the prostatic tissue showed only typical benign glandular hyperplasia. Four of the patients are living and well as long as eight years after prostatectomy and have given no clinical or roentgen evidence of carcinoma. Three patients died from other causes, and no metastatic lesions were found at autopsy. From the evidence presented in this study it seems clear that squamous metaplasia simulating carcinoma frequently occurs along the margin of the prostatic infarct. This condition should not be confused with the very rare squamous cell carcinoma of the prostate.

From Author's Summary.

LIVER NECROSIS FOLLOWING BURNS, SIMULATING THE LESIONS OF YELLOW FEVER. T. H. BELT, J. Path. & Bact. **48**:493, 1939.

Four patients with extensive superficial burns who died within four days presented severe damage of the liver with midzonal necrosis. Councilman lesions and intranuclear inclusion bodies. These findings were practically indistinguishable from those occurring in yellow fever.

FROM AUTHOR'S SUMMARY.

EMBOLIC MYOCARDITIS. S. DE NAVASQUEZ, J. Path. & Bact. **49**:33, 1939.

Myocardial lesions, the characters of which are defined, were encountered in 19 of 20 cases of subacute bacterial endocarditis (95 per cent). These lesions developed as a result of embolism of the coronary arteries or arterioles, the emboli being fragments of vegetations which varied in size and bacterial content. They were specific only so far as they showed evidence of embolism and polymorphonuclear cell reaction. There was no demonstrable relationship to the degree of cardiac failure, the cardiac valve affected, the character of the vegetation or the duration of the disease.

FROM AUTHOR'S SUMMARY.

SILICOSIS OF THE SPLEEN AND THE SILICOTIC NODULE. T. H. BELT, J. Path. & Bact. **49**:39, 1939.

The silicotic nodule in its mature form has the following distinctive characters: 1. There is a central focus of argentophil cellular remnants and dust. 2. There is a redundant avascular collagenous capsule which contains little or no dust. 3. There is a characteristic silicious ash after microincineration. 4. Though there is no definite quantitative correlation between the silica and the lesion, the silica content of the tissue as a whole is in excess of the normal range.

FROM AUTHOR'S SUMMARY.

BASAL CELLS OF THE CERVIX UTERI. R. CARMICHAEL and B. L. JEAFFRESON, J. Path. & Bact. **49**:63, 1939.

Small, poorly differentiated basal cells are present in patches, often exceeding 1 mm. in diameter, in the endocervical epithelium of almost all human cervices; they may occur either in the glands or at the surface of the canal and are usually more abundant in the upper half of the canal. They are apparently an undifferentiated product of the primitive cervical lining and probably act as reserve depots from which regeneration can take place after extensive destruction of the ordinary mucus-forming epithelium.

FROM AUTHORS' SUMMARY.

MYOCARDIAL SCARS. T. E. LOWE, J. Path. & Bact. **49**:195, 1939.

In a series of 5 cases the myocardial scars in the ventricles are reconstructed and described in detail. These scars are shown to have an anatomic position consistent with their being portions of one of the various muscle groupings of the ventricles. It is suggested that they represent the end stage of myocardial infarction due to interference with the blood supply to a portion of a muscle bundle, though this is not necessarily the result of coronary thrombosis or embolism.

FROM AUTHOR'S SUMMARY.

INTRAMESENTERIC DIVERTICULUM OF THE SMALL INTESTINE. J. MELLGREN, Virchows Arch. f. path. Anat. **302**:677, 1938.

A 15 cm. long intramesenteric diverticulum of the small intestine furnishes the basis of a thorough review of the literature relating to this anomaly and of a discussion of the theories advanced to explain the condition. Characteristic of such diverticula are their intramesenteric situation, their relative length, their gross and microscopic similarity to the normal intestine and their blind ending.

In the author's opinion, they are derived from the omphalomesenteric duct, but additional abnormality of development of the first part of the intestine must occur to account for the intramesenteric situation of the diverticulum.

O. T. SCHULTZ.

PARASITIC POSTERIOR REDUPLICATION OF THE HEAD. H. KAHRMANN, Virchows Arch. f. path. Anat. **302**:742, 1938.

Kahrmann describes 2 examples of a rare monstrosity characterized by the presence in the midline of the back of a reduplicated fetal head. The condition is the result of a second and incomplete invagination at the time of gastrulation.

O. T. SCHULTZ.

Microbiology and Parasitology

GROWTH AND METABOLISM OF TUBERCLE BACILLI. R. R. HENLEY and P. W. LEDUC, Am. Rev. Tuberc. **40**:313, 1939.

Studies were made of the growth, metabolism and tuberculin production of cultures of Mycobacterium tuberculosis, human type, PN strain, on mediums containing different proportions of glycerol and of nitrogen from different sources, chiefly asparagine, glycine and the ammonium salts of glutamic, malic and malonic acids. The production of acids was related to the proportion of glycerol present and to the source and proportion of nitrogen, but not to the amount of growth, nor to the rate of growth, nor to the amount of glycerol consumed. On mediums containing nitrogen at high levels cultures always became alkaline during the earlier stages of growth when the nitrogen was derived from asparagine, glycine, ammonium malate or ammonium glutamate. On malonate mediums under like conditions cultures first became acid; then, as the glycerol supply was depleted, the p_H values tended to rise. With the five sources of nitrogen mentioned, a definite relation was found between the glycerol-nitrogen ratio of the medium and the final reaction of the culture; the higher the ratio, the more acid the culture tended to become. With each of the five sources of nitrogen, the yield of bacteria obtained was dependent on, but not proportional to, the amounts of glycerol and nitrogen present. Yields approximating 2 Gm. of bacteria, dry weight, in 100 cc. of medium were obtained with each source. Under favorable conditions yields of 10 Gm. of bacteria were obtained from each gram of nitrogen, but under no conditions did the yield exceed 0.38 Gm. of bacteria per gram of glycerol. In mediums containing available nitrogen at high levels the glycerol supply was exhausted about the time the maximum weight was reached; at low levels glycerol continued to disappear after the bacteria stopped increasing in weight. The nitrogen metabolism in cultures on asparagine medium was studied. When they became acid, the fall in p_H value occurred about the time that the filtrate nitrogen was reduced to a minimum. No consistent relation was detected between the final p_H value and the residual ammonia in the filtrate. Potent tuberculins were obtained from cultures grown on mediums in which nitrogen was supplied by any of the five sources of nitrogen mentioned. The conclusion was reached that the nitrogenous constituents of the medium are the chief source of the alkali and that glycerol is the chief source of the acid, which is probably produced as an intermediate oxidation product and is in turn oxidized.

H. J. CORPER.

EXPERIMENTAL TRANSMISSION OF POLIOMYELITIS TO THE EASTERN COTTON RAT, SIGMODON HISPILUS HISPIDUS. C. ARMSTRONG, Pub. Health Rep. **54**:1719, 1939.

The first symptoms of poliomyelitis noted in the cotton rats were a roughened appearance of the fur and a tendency to react by violent jumping when agitated Flaccid paralysis developed in all animals which were considered affected. The

legs were paralyzed in all combinations, and respiratory difficulty developed in several, with the respiratory rate falling as low as 30 per minute in some. Two rats with respiratory failure died; the others were etherized. A number of other rodents were inoculated with the virus utilized in this study, including groups of Swiss mice, in which successive transfers were made, but no positive results were secured in animals other than the cotton rats. The eastern cotton rat is not vicious, and it multiplies readily in captivity. It is hoped, therefore, that when a sufficient supply becomes available and the most susceptible age is determined, the cotton rat may prove to be a cheap, convenient and useful laboratory animal for the study of poliomyelitis. It is conceivable, however, that the results secured may be due to some peculiarities of this particular strain of virus.

FROM AUTHOR'S SUMMARY.

INFECTIOUS POLYARTHRITIS OF RATS. W. A. COLLIER, J. Path. & Bact. **48**:579, 1939.

In Rattus norvegicus a spontaneous polyarthritis was observed, which was readily transmitted to white rats and field rats (Rattus brevicaudatus) by plantar, subcutaneous, intraperitoneal, intrapleural and intracerebral injections; characteristic polyarthritic symptoms ensued. For some rats the disease was fatal; others recovered, though in many of these the swelling of the joints persisted. All efforts to isolate a micro-organism of etiologic significance from the inflamed joints or from the organs have failed. On the other hand, bacteriologically sterile organs produced the disease on inoculation into other rats. The agent was found not only in the inflamed joints but in the blood, brain, lungs, pleural exudate, liver, spleen and peritoneal exudate. The agent was also present in animals in which no symptoms followed infection. Once the disease had been overcome there was distinct immunity to reinfection. Animals in which symptoms in the joints have not disappeared generally prove to be immune to subsequent inoculation, as do those which showed no signs of disease after the first injection. In no instance have complement-fixing, precipitating or neutralizing antibodies been discovered in the serum of immune rats.

FROM AUTHOR'S SUMMARY.

TUBERCLE BACILLI IN PERSONS CLINICALLY NOT TUBERCULOUS. A. SAENZ and G. CANETTI, Ann. Inst. Pasteur **62**:361, 1939.

Organisms were found directly, with difficulty or not at all in tissues obtained at autopsy from 76 tuberculous adults who clinically were called nontuberculous. The tuberculin test on the inoculated guinea pig, periodically repeated, was a more sensitive indicator of infection than cultures. Some four fifths of the caseous or calcified lesions, as well as some fibrous lesions, appeared to be sterile. Sterilization seemed to be a function of histologic maturity, depending thus on the site infected and on the age of the subject; older subjects in whom the rapidity of healing was retarded showed less sterilization. Sixty per cent of the tracheobronchial lymph nodes examined showed organisms, whether or not they appeared normal. The studies suggest that persistence of hypersensitivity in the adult is due not so much to continued virulence of the lesions of primary infection as to successive reinfections.

FROM AUTHORS' SUMMARY.

Immunology

A SKIN TEST FOR DETECTING GROUP C HEMOLYTIC STREPTOCOCCIC INFECTION CAUSING EPIZOOTIC LYMPHADENITIS IN GUINEA-PIGS. J. K. MOEN, J. Exper. Med. **64**:553, 1936.

A skin test with a crude bacterial extract prepared from group C (Lancefield) hemolytic streptococci was used as a means of detecting possible carriers of the

streptococcus causing epizootic lymphadenitis in guinea-pigs. A positive reaction similar to a positive tuberculin reaction was considered presumptive evidence of present or recent infection with this streptococcus. In 330 supposedly normal guinea-pigs 20 positive reactors were found. Over a period of fifteen months 195 negatively reacting animals used as a breeding stock yielded 1,296 progeny; none of the breeding stock or their progeny showed evidence of spontaneous lymphadenitis. Skin tests of 100 of the progeny were all negative. The use of this skin test as a means of obtaining breeding stock free from the streptococcus causing spontaneous lymphadenitis is suggested.

FROM AUTHOR'S SUMMARY.

THE SPECIFIC POLYSACCHARIDES OF TYPES I, II AND III OF PNEUMOCOCCUS. H. HEIDELBERGER, F. E. KENDALL and H. W. SCHERP, J. Exper. Med. **64**:559, 1936.

The thermolability of the specific polysaccharides of types I, II and III of Pneumococcus has been shown by three independent observations: (1) diminution of the viscosity of solutions on heating, (2) decrease in the amount of antibody precipitated from homologous rabbit antiserums and (3) increased tendency (S III) to pass through a collodion membrane. These effects may be explained most simply as a partial depolymerization under the influence of heat. In air, particularly in the presence of broth, oxidation also appears to be involved. Improved and simpler methods of preparation based on these findings are given for A I, S II and S III. The resulting products precipitate more anti-S from homologous rabbit antiserums than do the earlier preparations. The methyl glycoside of methyl galacturonate has been isolated from the hydrolytic products of A I and evidence of the ultimate structural unit obtained.

FROM AUTHORS' SUMMARY.

HYPERSENSITIVENESS AND ANTIBODY FORMATION IN TUBERCULOUS RABBITS. J. FREUND, E. H. LAIDLAW and J. S. MANSFIELD, J. Exper. Med. **64**:573, 1936.

Rabbits infected with bovine tubercle bacilli acquire hypersensitiveness to tuberculin, intracutaneously injected. This sensitiveness appears in the period from the second to the sixth week after infection and increases rapidly thereafter. Tests, as a rule, show fluctuation in the intensity of the sensitization. Sensitization is followed by an interval of several weeks preceding death during which the animals fail to react. Rabbits infected with bovine tubercle bacilli form antibodies that fix complement in the presence of tubercle bacilli. The antibodies appear after two weeks, increase during six to ten weeks and persist until the animals die. In the later period of infection the skin fails to react to tuberculin at a time when the serum contains complement-fixing antibodies.

FROM AUTHORS' SUMMARY.

LIPIDS AND IMMUNOLOGIC REACTIONS. F. L. HORSFALL JR. and K. GOODNER, J. Exper. Med. **64**:583, 1936.

Specific precipitates resulting from the interaction of type I antipneumococcus horse and rabbit serums and the homologous capsular polysaccharide have been analyzed by gasometric micromethods for total nitrogen, lipid nitrogen and lipid carbon. Lipid may, under certain conditions, form as much as 51 per cent or as little as 4 per cent by weight of the specific precipitates. The total lipid content of the specific precipitates, within the range studied, is entirely independent of the protein content. Lipid nitrogen forms a very small but detectable portion of the total nitrogen of the precipitates. The absolute lipid content of the precipitates is a function of the concentration of lipid in the reacting mixture prior to precipitation and seems to be governed by the laws of the phenomena of adsorption.

FROM AUTHORS' SUMMARY.

BLOOD-GROUPING FACTORS IN HORSES. V. A. HERMAN, J. Immunol. **31**:347, 1936.

From a study of the blood of eighteen horses Herman concluded that there are four blood groups corresponding to those of man. This similarity was established by absorbing horse serums with human 0 cells, thus removing the species-specific agglutinins, and observing a group-specific agglutination of human blood groups A and B and of the corresponding blood groups in horses. Similar results were observed by treating human 0 serums with horse erythrocytes, containing the horse species-specific agglutinogen 0, and then testing the serums against both human and horse red cells. The iso-agglutinins in horse serum also resembled those of man. The group-specific properties were also found in certain organs of the horse. The distribution of the blood groups in 910 samples of horse blood was found to be: group O, 10 per cent; group A, 41 per cent; group B, 16 per cent; group AB, 28 per cent. Five per cent of the samples could not be classified.

I. DAVIDSOHN.

PRECIPITIN AND COMPLEMENT FIXATION REACTIONS OF POLYSACCHARIDE EXTRACTS OF BRUCELLA. M. HIGGINBOTHAM and L. S. HEATHMAN, J. Infect. Dis. **59**:30, 1936.

The results of the precipitin tests with the polysaccharide preparations from seven strains of Brucella seem to show that organisms otherwise classified as of the same type may possess variable antigenic properties. The precipitin test is not satisfactory for establishing the type of a strain of Brucella, although extracts from three of the seven strains were found to give a positive reaction with the homologous antiserum only.

The results support the contention that the use of a polyvalent antigen embracing some local strains should be included in the routine serologic examination for undulent fever. All the serums from human beings with brucellosis which showed agglutination with the stock antigens (Brucella melitensis, Brucella abortus and Brucella suis) gave positive precipitin reactions with one or more of the polysaccharides of Brucella. Although the series is small the polysaccharide precipitin test seems to have no advantage over the agglutination test as a routine procedure and has the disadvantage of being impracticable because of the time and cost of preparing extracts.

The results of complement fixation tests with the polysaccharide extracts of Brucella and specific antiserums as well as of those with the extracts and patients' serums were not as clearcut as the precipitin reactions.

FROM AUTHORS' SUMMARY.

COMPLEMENT FIXATION IN NORMAL SERUMS TREATED WITH NUCLEI OF RED CORPUSCLES OF CHICKENS AND WITH TISSUE EXTRACTS. J. F. CHRISTENSEN, Ztschr. f. Immunitätsforsch. u. exper. Therap. **88**:325, 1936.

In active serums of chickens complement was fixed with nuclei of the chickens' own red corpuscles and of the corpuscles of other chickens. While group-specific properties were noted in the whole erythrocytes, only species specificness was found in the nuclei. The reaction did not take place at 0 C. and was absent when inactivated serum was used. Attempts to absorb the complement-fixing property with nuclear suspensions were unsuccessful in all but one serum. Inoculating chickens with nuclei and with red corpuscles did not increase the titers of the complement-fixing property of their serums. Immune serums produced in rabbits with the whole red corpuscles and with the nuclei of the red corpuscles of chickens brought out antigenic differences between the components of these cells. The active serums of chickens were later shown to fix complement with extracts of the chickens' own tissues and of those of different animals (duck, rabbit, sheep, calf and hog). The reaction with undamaged red cells of the rabbit and of the

sheep was very slight. Active serums of the sheep, horse and man were shown to possess the same ability to fix complement with different tissue extracts, and in the case of the serum of the rabbit inactivation at 56 C. did not remove the complement-fixing property, but heating at 62 C. destroyed it. The complement fixation with the normal serums is interpreted as a nonspecific "pseudoreaction." The necessity of a consideration of this phenomenon in complement fixation tests with active serums, and even with inactivated serum in the case of the rabbit, is obvious.

I. DAVIDSOHN.

VITAMINS AND IMMUNITY. H. J. JUSATZ, Ztschr. f. Immunitätsforsch. u. exper. Therap. **88**:472, 1936.

Rabbits that received food of sufficient calories but without vitamins showed a drop in the bactericidal properties of their blood and very poor precipitin response to injections of horse serum. Vitamin A did not improve the aforementioned properties. Small quantities of vitamin D raised the bactericidal properties of the blood but did not affect the antibody response, while large quantities influenced both properties adversely. The water-soluble vitamin B, in the form of yeast, had no influence on the bactericidal properties of the blood serum against staphylococci or on the formation of precipitin. Vitamin C, the natural and the synthetic product, as well as its sodium salt, effected a marked though transient increase of the bactericidal properties of the blood serum and enhanced greatly the production of precipitins, particularly if the vitamin was injected immediately preceding the injection of the horse serum or if it was mixed with it.

I. DAVIDSOHN.

Tumors

OBSERVATIONS ON RATS TREATED WITH SEX HORMONES. C. S. MCEUEN, Am. J. Cancer **36**:551, 1939.

The occurrence is reported of 3 tar cancers of the skin, 1 uterine cancer and 1 gastric cancer in rats fed with an estrogen obtained from the urine of pregnant women. A tar cancer in 1 control rat is recorded. Whereas in rats treated with estrone (theelin) for long periods carcinoma of the genital tract and breast occurred, accompanied by other, usual estrogenic effects, with prolonged injection of testosterone the females went into permanent vaginal diestrus, the pituitary weights were not above normal limits, somatic growth was not inhibited and the predominant neoplastic lesions were fibrous tissue tumors in areas of injections, some of which became sarcomatous.

FROM AUTHOR'S SUMMARY.

PULMONARY ASBESTOSIS. K. M. LYNCH and W. A. SMITH, Am. J. Cancer **36**:567, 1939.

During the past twelve years, in 2,343 consecutive necropsies Lynch and Smith encountered 7 cases of primary carcinoma of the lung, including 1 instance of this lesion in an early stage, discovered only on histologic examination, and 2 cases of asbestosis. Among these necropsies were 35 showing some degree of asbestosis; in the majority of instances this was a minor finding and not the cause of illness or death; in a few the condition was advanced. If all necropsies and all cases of cancer of the lung are included, the incidence of primary pulmonary carcinoma in this necropsy service over this period was 0.3 per cent. If the 35 cases in which asbestos deposits were shown and the 2 cases of cancer of the lung in association with asbestosis are omitted, the incidence was 0.21 per cent. Among the cases of asbestosis (35) the incidence of carcinoma of the lung (2 cases) was approximately 6 per cent. Whether this is to be taken as of significance, especially in comparison with the general rate, is questionable. The series of cases of asbestosis is small and the possible statistical error great. It has seemed desirable to record,

in addition, the observation that advanced asbestosis may lead to bronchial epithelial metaplasia of a type encountered in other locations where cylindric epithelium may give rise to squamous cell carcinoma.

FROM AUTHORS' COMMENT.

MYELOID LEUKEMIA AND NONMALIGNANT EXTRAMEDULLARY MYELOPOIESIS IN MICE. W. A. BARNES and I. E. SISMAN, Am. J. Cancer **37**:1, 1939.

Eight transmissible strains of myeloid leukemia that have been studied possessed characteristics which with rare exceptions remained unaltered in the course of successive subpassages. Transmission experiments indicated the neoplastic nature of these disturbances. The malignant cells in 5 cases were myelocytes and in 3 cases were myeloblasts maturing into promyelocytes or myelocytes. Although morphologically the individual cells of these leukemias with a single exception resembled normal cells, they differed from the latter as well as from one another in their behavior in inoculation experiments. These differences included ability to produce tumors, color of the leukemic infiltration, localization in various tissues and transmissibility to different stocks of mice. The myelocytes characteristic of one strain underwent mitotic division in tissue cultures and, like malignant cells, failed to mature. In 6 additional cases of myeloid leukemia attempted transmissions to mice that were not highly inbred were unsuccessful. The infiltrations in nonmalignant extramedullary myelopoiesis in mice may be as extensive as those in myeloid leukemia. Nonmalignant medullary myelopoiesis is frequent in apparently healthy old mice of one of the stocks studied. It often accompanies suppurative inflammations, particularly those of long standing, and spontaneous and transmitted neoplasms. The differentiation of nonmalignant extramedullary myelopoiesis from leukemia is occasionally difficult. Features of the nonmalignant disturbance include conspicuous maturation of myeloid cells, association with erythrogenesis, presence of megakaryocytes, absence of epicapsular and tumor-like infiltrations and failure of transmission to other mice. Parenteral administration of suspensions of Bacillus coli is a simple procedure to stimulate extramedullary hemopoiesis in mice. Exposure of mice with spontaneous mammary tumors to small doses of roentgen rays did not produce myeloid leukemia and failed to increase the extent of nonmalignant extramedullary myelopoiesis.

FROM AUTHORS' SUMMARY.

INTRACEREBRAL CARCINOMATOUS METASTASES. C. C. HARE and G. A. SCHWARZ, Arch. Int. Med. **64**:542, 1939.

Bronchogenic and mammary carcinoma commonly metastasize to the brain. In cases of cerebral metastasis the primary carcinoma is most commonly in the lung or the breast. In our series of 100 cases there were 65 in which the primary tumor was so located. A bronchogenic carcinoma often becomes manifest in the effects of a cerebral metastasis before there are any pulmonary signs. Carcinoma with cerebral metastases is not uncommon in persons less than 40 years of age. In our series it occurred in 27 of 100 such persons. The disease occurs predominantly in male patients, in the ratio 3.2:1, provided the cases of primary carcinoma of the breast are excluded. Symptoms of metastasis are usually of short duration before the patient becomes seriously ill. A gradual onset of intracranial symptoms in cases of metastatic intracerebral carcinoma, according to this study, is infrequent (17 per cent). The onset occurred suddenly in 36 of these 100 cases. Patients with metastatic cerebral tumor do not tolerate surgical procedures well. The average duration of life from the time of the first neurologic symptom until death was three and six-tenths months for the 32 persons who died while under the authors' care. The period of survival was much shorter for those who were operated on.

Signs of chronic debilitating disease may be absent. They were lacking in 40 per cent of the cases in this series. Abnormality of the spinal fluid is a

prominent finding. It occurred in 70 per cent of the cases in which the spinal fluid was examined. Roentgenographic erosion of the sella turcica may be present. In this series it was not uncommon, in spite of the supposedly short duration of the cerebral metastases. Encephalographic and clinical studies may localize one of the metastatic masses, which is usually the largest, and may fail to show the presence of other, smaller nodules. Even after metastasizing to the brain bronchogenic carcinoma may not appear as such on roentgenograms of the chest. Metastatic cerebral tumor may be single or multiple. Multiple tumor was observed in 20 brains removed at autopsy; single nodules, in the remaining 14. In practically all cases of cerebral metastasis there are metastases to other organs. Cerebral disease in addition to the metastases may be present. In 1 of the cases a cholesteatoma was observed in the cerebellopontile angle at autopsy; in 3 syphilis of the central nervous system was evidenced by a positive Wassermann reaction of the spinal fluid. Surgical removal of single metastases may in a few cases prolong life for months or even for several years; such cases, however, form a small percentage of those in which operation is done, most of the patients dying shortly after craniotomy. Subtemporal decompression often relieves the headache and affords great comfort to the patient and to his relatives. When craniotomy is to be performed, roentgen studies of the chest should be made regardless of the age of the patient.

FROM AUTHORS' SUMMARY.

CARCINOMA OF THE BODY AND TAIL OF THE PANCREAS. G. L. DUFF, Bull. Johns Hopkins Hosp. **65**:69, 1939.

An account of the pathologic features of carcinoma of the pancreas in general is followed by a description of the various modes and directions of the spread of cancer of the body or of the tail of the pancreas based on observations at autopsy in 16 cases. A comparison with the observations at autopsy in 16 consecutive cases of carcinoma of the head of the pancreas shows that primary cancer of the body or of the tail of the pancreas tends to spread much more widely and massively than carcinoma originating in the head. An adequate explanation of this tendency is afforded by the anatomic position and relations of the body and tail as contrasted with the head of the pancreas. Direct extension of carcinoma of the body or of the tail of the pancreas frequently leads to widespread involvement of the peritoneum and sometimes to invasion of the stomach or intestines. The abdominal lymph nodes are often massively involved. Invasion of the splenic vein with the consequent occurrence of massive metastasis to the liver may be followed by occlusion of the splenic vein, obstruction of large intrahepatic branches of the portal vein or even occlusion of the portal vein itself.

FROM AUTHOR'S SUMMARY.

FROG CARCINOMA IN TISSUE CULTURE. B. LUCKÉ, J. Exper. Med. **70**:269, 1939.

The adenocarcinoma of the leopard frog may be cultivated with ease in plasma mediums. In such cultures two types of growth occur with regularity. The first is in the form of tubules which promptly grow out in the solid medium and retain their tubular form as long as they remain completely enveloped by plasma. When, however, they make contact with the surface of the glass, they adhere to it, the part in contact becomes flat, and its cells now grow no longer as tubules but as membranes.

FROM AUTHOR'S SUMMARY.

INHIBITION OF TRANSPLANTABLE TUMOR BY TISSUE EXTRACTS. D. A. MACFADYEN, E. STURM and J. B. MURPHY, J. Exper. Med. **70**:475, 1939.

Mammary tissue of pregnant rabbits is found to contain an agent which inhibits growth of Bashford adenocarcinoma 63 in mice, but this material is without effect on Crocker sarcoma 180. Of the tissues so far studied, the mammary gland of the rabbit has yielded the most active product. From the results it

appears probable that pregnancy enhances the production of the agent, but this cannot be considered as established beyond doubt. The factor is found, with diminished activity, only in the protein fraction obtained from the aqueous extract by full saturation with ammonium sulfate. (See also D. A. MacFadyen and J. B. Murphy [*J. Exper. Med.* **70**:461, 1939].) FROM AUTHORS' SUMMARY.

ISOLATION OF RABBIT PAPILLOMA VIRUS PROTEIN. J. W. BEARD, W. R. BRYAN and R. W. G. WYCKOFF, J. Infect. Dis. **65**:43, 1939.

A protein free from carbohydrate and detectable by the Molisch test has been isolated by differential ultracentrifugation from extracts of infectious warts of cotton-tail rabbits. It gives a sediment with the sharp boundary characteristic of an undamaged molecular species and has a principal sedimentation constant $s_{20} = ca\ 250 \times 10^{-13}$ cm. sec^{-1} $dynes^{-1}$.

The yield of this protein was proportional to the activity of the virus in the extracts from which it was derived, varying from 1.0 mg. per gram of very infectious warts to 0.008 mg. per gram of warts of low infectivity. No protein has been obtained from noninfectious warts of domestic rabbits. The infectivity associated with the heavy protein was constant and was not related to the infectivity of the source extracts. Papillomas were consistently induced by the inoculation of 10^{-9} Gm. of protein.

Changes in the protein were accompanied by changes in the activity of the virus. Infectivity was destroyed when the protein was coagulated at 65 to 66 C. or broken down by the action of acid or alkali. Virus infectivity was removed from the solution by precipitation of the protein at its isoelectric point and was associated with the precipitate under these conditions.

FROM AUTHORS' SUMMARY.

CANCER OF THE THYROID GLAND. J. DEJ. PEMBERTON, Surg., Gynec. & Obst. **69**:417, 1939.

A series of 774 cases of cancer of the thyroid was reviewed; in 517 the diagnosis was established by microscopic examination; in the remainder the disease was obvious from clinical observation alone. The age incidence corresponds to that of carcinoma elsewhere, although the occurrence of this type of cancer in children is more common than is generally suspected. The sex incidence shows a ratio of 1 male to 1.74 females, whereas the ratio for benign nodular goiter is 1 to 5.07. In a large percentage of cases carcinoma of the thyroid originates in a benign adenoma, thus stressing the importance of prevention and treatment of lesions of the thyroid. The basal metabolic rate is inconstant and of no diagnostic aid in determining the presence of a malignant change in the thyroid. Sixty-eight per cent of the tumors were graded as 1 or 2 in regard to malignancy, based on anaplasia and dedifferentiation. Increased interest on the part of the pathologist is shown by the steady rise in the percentage of cancers discovered in patients operated on for tumors considered benign. The author classifies malignant neoplasms of the thyroid as: papillary adenocarcinoma, adenocarcinoma in adenoma (malignant adenoma), diffuse adenocarcinoma and sarcoma. The distinguishing clinical features of papillary adenocarcinoma are the low grade of malignancy, marked radiosensitivity and tendency for the lesion to spread to regional lymph nodes, where it may be confined without further dissemination for many years. For these reasons radical removal and postoperative irradiation of the site offer a good chance of cure. Characteristic of adenocarcinoma in adenoma are the low grade of malignancy and tendency to early dissemination by way of the blood stream. Because of the lack of early involvement of lymph nodes and the invasion of blood vessels, the presence of cervical metastases of this type has a grave prognostic significance. Diffuse adenocarcinoma of the thyroid is commonly of higher grades of malignancy than the other types and behaves like such carcinoma elsewhere. Both squamous carcinoma and sarcoma of the thyroid are rare and very

malignant. Metastases from thyroid carcinoma are most frequent in the cervical lymph nodes and next in the lungs. The percentages of persons with malignant tumor of the thyroid who have lived three, five and ten years or more after treatment are 77, 70 and 58, respectively.

FROM AUTHOR'S SUMMARY. (WARREN C. HUNTER)

PAGET'S DISEASE OF THE NIPPLE. R. MUIR, J. Path. & Bact. **49**:299, 1939.

The examination of 42 cases of Paget's disease of the nipple fully supports the view that the lesion of the nipple is due to intraepithelial growth of cancer cells which have spread from a malignant growth in the upper extremity of a lactiferous duct—intraduct carcinoma. It also supports the view formerly expressed as to the association of Paget's disease with carcinoma of the mamma. In all the cases intraduct carcinoma was present in the nipple. Of 39 cases fully examined, it was present in both breast and nipple in 34; in 30 it was accompanied by infiltrating carcinoma of the breast. The carcinoma of the breast which is so often associated with Paget's disease is ordinarily due to extension from an independent focus of intraduct carcinoma in the breast. In view, however, of the mode of spread of intraduct carcinoma, the possibility of carcinoma of the breast resulting from a downward direct extension of intraduct carcinoma in the nipple must be admitted, but no undoubted example of this has been met with in this series of cases. On the other hand, in 6 cases of carcinoma of the breast there was found an upward spread of intraduct carcinoma from a level below the nipple, and this had just reached, or was just reaching, the epidermis at the appearance of the clinical signs of Paget's disease. The time of appearance or the stage of Paget's disease gives no information as to the presence or the absence of carcinoma in the breast. The cases recorded here show a high frequency of carcinoma of the breast and emphasize the grave significance of the lesion of Paget's disease.

FROM AUTHOR'S SUMMARY.

SQUAMOUS CELL CARCINOMA OF THE TONSIL IN THE DOG. F. W. WITHERS, J. Path. & Bact. **49**:429, 1939.

Although references to tonsillar carcinoma in the dog are extremely rare in the literature, observations at the Royal Veterinary College, London, indicate a comparatively high incidence of the condition among town-kept dogs. During the period of sixteen months covered by the cases under review approximately 20,000 dogs were presented for examination in the clinics of the college and, of those that died or were destroyed, 396 were examined post mortem. Among these there were 100 with neoplasia, of which 53 had carcinoma of various kinds and, of the latter, 24 had tonsillar epithelioma. The incidence is therefore approximately 1.2 per thousand of all dogs presented for examination, 6 per cent of all dogs examined post mortem and 24 per cent of all dogs with neoplasia. There were 10 dogs with squamous carcinoma in sites other than tonsillar, and of these 8 showed the skin and 2 the urinary bladder affected. Only 1 animal of a species other than the dog was recorded as having carcinoma of a tonsil during this period, namely, a cat, in which the primary lesion involved the tonsil, the soft palate and the root of the tongue, with a secondary deposit in the local lymph node; it was impossible to determine exactly the primary site of origin. The condition appears to be commonest in dogs from 6 to 10 years of age, which is roughly the equivalent of 40 to 70 years in man.

FROM AUTHOR'S DISCUSSION.

EXPERIMENTAL EPITHELIOMA OF THE STOMACH. A. BESREDKA and L. GROSS, Ann. Inst. Pasteur **62**:253, 1939.

The stomach of the rabbit shows pronounced receptivity to the Brown-Pearce epithelioma. The appearance of the epithelioma in the stomach is preceded by a

short period of incubation. The tumor appears as a more or less voluminous growth localized at the point of inoculation; in about half the cases metastases occur in the internal organs. Rabbits with resorbed intracutaneous tumors of this type show solid immunity to inoculation of the Brown-Pearce epithelioma in the stomach.

FROM AUTHORS' SUMMARY.

ADENOMYOMA OF THE GALLBLADDER. P. EISERTH, Virchows Arch. f. path. Anat. **302**:717, 1938.

Thirteen cases of adenoma, or better adenomyoma, of the gallbladder were encountered in a total of 4,000 necropsies. Of the two views as to the origin of adenomyomas—that it concerns congenital maldevelopment or that it results from chronic inflammation—the author accepts the former. A relationship between adenomyoma, chronic cholecystitis and cholelithiasis was not observed, although the cystically enlarged glands may become the site of intramural concretions. Transition of adenomyoma to adenocarcinoma was observed in 1 instance.

O. T. SCHULTZ.

Medicolegal Pathology

SUBARACHNOID HEMORRHAGE OF ANEURYSMAL ORIGIN: ITS INTEREST IN LEGAL MEDICINE. F. THOMAS, Ann. d'anat. path. **13**:969, 1936.

The article is a review of the subject of cerebral aneurysms. The literature, comprising 72 references, is well covered. A large number of photographs of gross specimens are included, demonstrating many varieties of cerebral aneurysms and malformations of the basilar cerebral arteries. The etiologic varieties are discussed, i. e., arteriosclerotic, syphilitic, mycotic-embolic and congenital. The clinical factors relating to medicolegal problems are covered. In the discussion of congenital aneurysms emphasis is placed on the observation of the lesion in the younger age group and the absence of trauma or of antecedent disease.

PERRY J. MELNICK.

CONGENITAL HEART DISEASE ASSOCIATED WITH SUDDEN DEATH. S. LYSS, Deutsche Ztschr. f. d. ges. gerichtl. Med. **31**:248, 1939.

The cases discussed in this article were encountered in 20,000 postmortem examinations made at the Hafenkrankenhaus, Hamburg, from 1901 to 1938. The anomalies were grouped as (*a*) defects responsible for death, (*b*) defects incidental to death and (*c*) patent foramen ovale. The latter group was studied intensively only for the years 1936 and 1937. There were 11 cases in the first group, and in most of them septal defects and various distortions of the heart chambers were presented. When they occurred in children, they were noted during the first few hours or days of life, although their occurrence in stillborn infants was also observed. The only adult showing such an anomaly was a man 21 years old, who had pulmonary stenosis.

The second group included 22 persons with incidental cardiac and vascular defects which fell into the following categories: (*a*) ventricular septal defects, (*b*) atresia of the aortic isthmus, (*c*) aortic stenosis, (*d*) anomalies of valves and (*e*) patent ductus Botalli. This group contained a preponderance of adults except in the last division, in which there were 4 adults and 4 children.

Patent foramen ovale was found 78 times in 2,015 autopsies, an incidence of only 3.5 per cent. In 2 of the cases it was associated with paradoxic embolus. The low percentage is at variance with the observations of Aschoff, who placed the incidence between 20 and 30 per cent.

GEORGE J. RUKSTINAT.

TRAUMATIC ALTERATION OF THE ELASTIC FIBROUS SYSTEM OF THE LUNG. S. OKRÖS, Deutsche Ztschr. f. d. ges. gerichtl. Med. **31**:308, 1939.

Okrös reported a comparative study of the elastic fiber system of the lung in the presence of a gunshot wound, a stab wound, a tear or compression made during life and post mortem. Somewhat similar changes took place in the elastic fibers with all of the aforementioned traumas when these were inflicted during life, but the changes were most marked after gunshot wounds. Characteristically, the fibers contract near the site of injury and form a dense, feltlike zone. At a distance of 6 to 15 alveoli removed from the traumatized zone the elastic fibers are torn, frayed or curled into ball-like contractures. All of these changes are lacking when the wounds have been made post mortem.

GEORGE J. RUKSTINAT.

Technical

MITOTIC LEUKOBLASTS IN THE PERIPHERAL BLOOD IN INFECTIOUS MONONUCLEOSIS. H. BOWCOCK, Am. J. M. Sc. **198**:384, 1939.

In infectious mononucleosis, mitotic leukoblasts and other unusual cell forms may appear in the peripheral blood near the peak of the leukocyte count. The occurrence of such immature and unusual cell forms is emphasized in order that confusion may be avoided in the diagnosis of this relatively benign illness, which often resembles acute leukemia in many respects. FROM AUTHOR'S SUMMARY.

POLARIZED LIGHT FOR THE STUDY OF MYELIN DEGENERATION. C. O. PRICKETT AND C. STEVENS, Am. J. Path. **15**:241, 1939.

A study has been made of the merits of the polarized light method as compared with the Marchi and sudan III methods of demonstrating myelin degeneration due to transection of the peripheral nerves in the rat. The polarized light method was found to be rapid and accurate. The changes depicted were consistent and did not depend for their demonstration on numerous technical manipulations. Changes in both myelin sheaths and axis-cylinders were visible in the same preparation. As compared with the polarized light method the Marchi method gave very inconsistent results. The sudan III method was consistent but failed to reveal the early changes following transection. Marked and advanced changes were shown by the polarized light method in nerves which had been transected only twenty-four hours. The earliest degeneration shown by the Marchi method was shown seventy-two hours after transection, and the earliest by the sudan III method, one hundred and twenty hours after transection. The thickness of the section influenced the structural detail observed by the polarized light method. Sections 10 microns in thickness showed more detail than sections which were thicker. It was found advantageous to uncross the analyzer prisms in determining the continuity of fibers which appear segmented, for distinguishing between edema of axis-cylinder and periaxillar accumulation of isotropic material and for revealing isotropic fibers masked by the crossed prisms. Sections of unfixed fresh normal or degenerating nerves when viewed by polarized light presented an appearance considerably different from that of fixed nerves. This does not detract, however, from the usefulness or reliability of the method.

FROM AUTHORS' SUMMARY.

A MORE RAPID METHOD OF GUINEA PIG INOCULATION FOR THE DIAGNOSIS OF TUBERCULOSIS. C. I. WOOLSEY, J. Lab. & Clin. Med. **24**:855, 1939.

The average length of time required for the diagnosis of tuberculosis by intracutaneous injection of the exudates into guinea pigs is three weeks, in comparison with the six to eight weeks required by the formerly used subcutaneous route. Of the 173 guinea pigs used for injections in this series, only 5 died before the presence

or the absence of tubercle bacilli in the fluid could be demonstrated. Since the diagnosis is made from the initial lesion and substantiated by observation of regional adenopathy and by autopsy, there is little chance of a spontaneously infected guinea pig not being recognized as such. Those guinea pigs that remain uninfected for six weeks may be kept as breeding stock. FROM AUTHOR'S SUMMARY.

ARTIFICIAL AND SERUMLESS MAINTENANCE MEDIUMS. L. E. BAKER and A. H. EBELING, J. Exper. Med. **69**:365, 1939.

Several mediums designed for maintaining the life of cells and of organs outside the body are described. Cultures made from a pure strain of fibroblasts have been maintained in these mediums in vital condition and with little or no proliferation for periods varying from forty-three to fifty-six days. One of these mediums is very simple, inexpensive and easy to prepare; and one is serumless.

FROM AUTHORS' SUMMARY.

Society Transactions

AMERICAN SOCIETY FOR EXPERIMENTAL PATHOLOGY

ERNEST W. GOODPASTURE, *President*

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Twenty-Seventh Annual Meeting, New Orleans, March 13-17, 1940

Preliminary Observations on Rabies in the Chick Embryo. JAMES R. DAWSON JR., Vanderbilt University, Nashville, Tenn.

Chick embryos have been infected by intracerebral inoculation of fixed virus, street virus and human virus of rabies, each of which produces typical Negri bodies throughout the nervous system of the embryo. One strain which has been maintained in chick embryos for forty-seven generations has exhibited profound alterations in its virulence for rabbits. Intracerebral inoculation of this chick embryo–adapted virus in rabbits is followed by development of clinical evidences of acute encephalitis in the form of fever, incoordination and weakness. Of 19 animals treated in this manner, however, 13 survived; following this mild self-limited disease they disclosed active immunity to the virus of rabies and were able to withstand an intracerebral injection of about 1,000 minimal lethal doses of either fixed virus or mouse brain street virus. Less dramatic alterations appear to have occurred in the pathogenicity of this strain for dogs and mice.

Sputum Studies as an Aid to Prognosis in Cases of Pneumonia. ARTHUR W. FRISCH, Wayne University, Detroit.

The number of extracellular encapsulated pneumococci per oil immersion field as determined from smears of rusty sputum treated with Wright's stain was significantly related to the incidence of bacteremia, leukopenia, multiple lobar involvement and mortality rate in 250 proved cases of pneumonia. Prognoses based on such counts were accurate enough to be of value in the selection of cases of potentially severe pneumonia both on admission of the patients to the hospital and during the course of therapy.

Immunochemistry of Catalase: III. Langmuir Multilayers with Serum Globulin. Lyman Fourt, University of Chicago.

Using crystallized catalase as antigen and Langmuir's technics for the measurement of molecular dimensions, I have compared whole immune or normal serum and the corresponding globulins with respect to reaction in multilayers.

After an initial deposit has been made, repeated treatments with one substance produced little change. Starting from catalase, whole immune serum and its globulin fraction give nearly the same sequences of thickness changes: catalase, 50 angstrom units; anticatalase, 50 units; then catalase, 5 units, alternating with anticatalase, 50 units, to more than ten layers. An initial layer of serum is 25 units, while an initial layer of globulin is 70. Either the globulin molecules are differently oriented in each mode of adsorption, or the proportion of globulins varies, being least in the 25 unit deposit from whole serum. Anticatalase and catalase layers alternate from an initial layer of immune serum, but the first few

deposits are less thick than in the other series; some other systems fail to go on above the serum, although continuing well from the antigen.

Specificity appears clearer cut with whole serums than with globulin fractions. Normal serum does not alternate with catalase; normal globulin deposits on catalase (25 angstrom units), but this nonspecific adsorption fails to continue. Serum globulin and whole serum of the same or different species alternate, resembling specific pairs.

Natural Quantitative Respiratory Contagion of Tuberculosis. WILLIAM F. WELLS and MAX B. LURIE, University of Pennsylvania, Philadelphia.

By means of the Wells air centrifuge, it is possible to determine quantitatively the number of tubercle bacilli suspended in a given volume of air by collecting the bacilli in a known volume of glycerin broth and seeding aliquot portions thereof on a modified Löwenstein egg medium. In an apparatus for the study of experimental air-borne disease rabbits were exposed to measured quantities of air-borne tubercle bacilli. The number of bacilli cultured from sample rabbits' lungs corresponded to the dose computed from the quantity of air breathed by these rabbits and the number of bacilli cultured from sample volumes of this air.

Rabbits which inhaled 1,000 or more tubercle bacilli died in a period of five to six weeks with massive nodular caseous pneumonia, without tuberculosis of any other organ, irrespective of whether they were naturally highly resistant or highly susceptible to tuberculosis. With smaller numbers of inhaled bacilli the natural resistance of the rabbit exposed played a determining role. When less than 100 units of bacilli was inhaled, the naturally susceptible rabbits died of massive caseous pneumonia with extensive caseation of the draining tracheobronchial lymph nodes and destructive lesions of hematogenous origin in the different organs—a picture of first infection resembling the childhood type of tuberculosis in man. Under the same conditions of exposure resistant animals developed a slowly progressive localized ulcerative pulmonary phthisis in which the draining lymph nodes and the internal organs were spared—a disease resembling the reinfection type of adult pulmonary tuberculosis in man.

The death of 96 per cent of tubercle bacilli suspended in the air was demonstrated when the air was exposed for three seconds to ultraviolet rays in the 2,500 to 3,700 angstrom wave band, as demonstrated by culture, inoculation in guinea pigs and inhalation by rabbits.

Comparison of Urinary and Serum Proteins. WILLIAM A. MURRILL, M. H. SOULE and L. H. NEWBURGH, University of Michigan, Ann Arbor.

Urinary protein antiserum and total serum protein antiserum were prepared by injecting into rabbits the unaltered antigens from a patient with nephritis. By means of the precipitin reaction it was determined that these two types of antiserum reacted equally well with both homologous and heterologous antigens. Specimens of both types of antiserum were then adsorbed with total serum protein and the filtrates subsequently tested for the presence of specific urinary protein precipitins. The serum protein completely adsorbed all of the antibodies specific for both urinary and serum proteins, indicating that there are no antigenically active substances in the urine which are not present in the serum. In a like manner, samples of both types of antiserum were adsorbed with urinary protein and the filtrates tested for the presence of specific serum protein precipitins. The urinary protein antiserum gave negative reactions, whereas in the serum protein antiserum antibodies of high titer against total serum protein were demonstrated. The failure of urinary protein to adsorb completely the antibodies against total serum protein indicates that urinary protein is a fraction of total serum protein. These results support the classic view that serum protein is made up of two or more independent entities.

Metamorphosis of the Nucleus of the Neuron in Inanition Due to Prolonged Starvation. WARREN ANDREW, Baylor University, Dallas, Texas.

Several persons who died in an extremely emaciated condition showed marked degeneration of the nerve cells in the cerebral cortex with less striking changes in the cerebellum.

Sections were stained by methods to be described. The cytoplasmic changes consisted of chromatolysis, vacuolation and final complete dissolution of the cell substance. The nuclear changes included migration of the nucleolus to an eccentric position (in the majority of cells) and great accumulation of basophilic granules in the nucleus, causing the nuclei to resemble those of the larger glial cells.

In all the cases extensive destruction of cells and neuronophagia were seen.

Experimental work on mice of different ages, which were starved for varying periods, has confirmed, in general, the conclusions as to the nature of the nerve cell changes brought about by inanition.

Many of the changes caused by inanition in mice and in men are similar to those occurring in senility. Age, therefore, is a factor which must be taken into account in studies of this type.

The Pathologic Nature of Irradiation Sickness; A New Method for Inducing Shock. VIRGIL H. MOON, KARL KORNBLUM and DAVID R. MORGAN, Jefferson Medical College, Philadelphia.

Irradiation sickness was produced in dogs in order to make observations on the hemoconcentration and on the visceral changes. From 1,400 to 2,800 roentgens was given in divided doses over different parts of the abdomen. After an interval of sixty to seventy-two hours, severe illness developed, and hemoconcentration, ranging from 15 to 50 per cent, appeared. Urination was decreased, and blood was seen in the urine, feces and vomitus. The illness progressed rapidly, and death occurred by circulatory failure within twenty-four hours.

The gross and microscopic features were those characteristic of shock. They included capillovenous congestion of the viscera, petechial hemorrhages in mucous and serous surfaces, edema of the lungs and of the mucosae, and parenchymatous degeneration of the liver and kidneys. The results were concordant in 12 animals so treated.

An additional feature was degeneration, progressing to necrosis, of the gastrointestinal mucosa. The epithelium lining the crypts and covering the villi showed all stages of disintegration. Some crypts contained nuclear and cellular debris; others were denuded and empty. Some of the villi retained their epithelial covering while that in the crypts was necrotic and disintegrating.

Our results corroborate those of Whipple and others: Roentgen radiation of high voltage causes delayed necrosis of the intestinal mucosae, accompanied by shocklike manifestations. Apparently the latter are due to absorption of material from the damaged mucosa. The condition is accompanied by progressive hemoconcentration, and the visceral changes observed post mortem are characteristically those of shock.

These results provide experimenters with a new method for inducing shock by causing damage to the tissues. This method eliminates pain and emotional responses, sympathoadrenal hyperactivity, hemorrhage and anesthesia, conditions which are assigned as causes for shock by some writers.

Effect of "Sex Hormones" on the Formation of Immune Bodies. E. VON HAAM and IRENE ROSENFELD, Ohio State University, Columbus.

In a series of experiments the influence of the administration of various doses of "sex hormones" on the development of agglutinating and protective antibodies against a laboratory strain of type I pneumococci was studied in rabbits. In

other experiments the mechanism of the protective action of these substances against lethal doses of the same organism was studied in mice. A certain quantitative correlation between the amount of such a substance injected and the amount of antibody formed could be demonstrated, and optimal immunizing or protective doses could be determined. Excessive doses of these substances proved definitely injurious to the development of antibodies. The role played by the interval between the administration of the substances and the production of the infection was investigated.

Geographic Distribution of Poliomyelitis in Louisiana. ALBERT E. CASEY and BRANCH J. AYMOND, Louisiana State University and State Board of Health, New Orleans.

Through the application of biometric procedures, especially of an objective method for detecting even the smallest epidemic, detailed and controlled studies on epidemic and sporadic poliomyelitis in Louisiana have been made possible. The relations of epidemic and sporadic poliomyelitis to geography, season, size of community, water supply, sewage systems and host factors, such as age, sex and race, are considered, and a comparison with other diseases is made.

Effect of Certain "Sex Hormones" on the Spread of India Ink and Resistance to Virus Infection. DOUGLAS H. SPRUNT and SARA MCDEARMAN, Duke University, Durham, N. C.

Last year we reported work which showed that certain endocrine states affect the spread of india ink in the rabbit's skin and also change the animal's resistance to infection with the virus of vaccinia. We have extended this work and can now report further studies. We find that the increased spread of india ink resulting from castration persists for a long period of time and that injected testosterone propionate has no effect on this spread. Injected estrogen, however, decreases the spread of india ink in castrates, male and female rabbits, and increases their resistance to infection with the virus of vaccinia.

Effect of Temperature and of Starvation on the Character of Growth of Frog Carcinoma Implanted in the Anterior Chamber of the Eye. BALDWIN LUCKÉ and H. SCHLUMBERGER, University of Pennsylvania, Philadelphia.

The adenocarcinoma which commonly occurs in the kidneys of leopard frogs is readily transplantable into the anterior chamber of the eye, where it develops according to well defined structural patterns. The manner and rate of such growth may be clearly observed over long periods through the slit lamp microscope and may be recorded objectively by photographs. This technic has been applied in the present study, which deals with the effect on tumor growth of two environmental factors, temperature and starvation. It was found that starvation of such a degree as led to great emaciation of the animals affected neither the manner nor the rate of growth. In groups of frogs maintained for months at two temperatures, 40 F. apart, the tumors grew somewhat more rapidly at the higher temperature, but there were no discernible effects of temperature on the manner of growth. It is concluded, therefore, that in a cold-blooded vertebrate, the frog, tumor growth is affected only to a minor degree by temperature and by starvation.

Glycolipoid Antigen of Meningococcus and Gonococcus. ALDEN K. BOOR and C. PHILLIP MILLER, University of Chicago.

Additional observations have been made on the factors influencing the demonstration of the glycolipoid antigen (Boivin) in the meningococcus and the gonococcus.

Pathologic Changes in Experimental Gonococcic Infection. C. PHILLIP MILLER and WALTER D. HAWK, University of Chicago.

The pathologic changes in the organs of mice intraperitoneally inoculated with gonococci suspended in mucin are described and compared with those resulting from the injection of sterile mucin alone.

Changes in the Distribution of Renal Phosphatase in Experimental Nephropathies. OPAL E. HEPER, J. P. SIMMONDS and HELEN GURLEY, Northwestern University, Chicago.

The phosphatase content of the kidneys of dogs was determined quantitatively by Bodansky's method, and its distribution in the renal units, by Gomori's microtechnical method. In normal dogs the phosphatase is distributed in a narrow zone along the free margin, i. e., next the lumen, of the cells lining the proximal convoluted tubules. Poisons that induce degenerative changes in the epithelium of these tubules alter the distribution of phosphatase in the cells. The earliest change is a broadening of the band of phosphatase in the marginal portion of the cells. When actual necrosis has occurred, the phosphatase is diffused uniformly throughout the necrotic cell mass. It still stains, but less deeply, apparently not because of any material reduction in amount but rather because of wider distribution through the entire cell instead of along a narrow marginal zone. Chemical tests revealed little alteration in the quantity of phosphatase present in kidneys, even in those with extensive necrosis. The relation of this change to calcification in the renal tubules in experimental nephropathies is discussed.

Virus of Lymphocytic Choriomeningitis in Man. MARION E. HOWARD, Yale University, New Haven, Conn.

Several strains of the virus of lymphocytic choriomeningitis have been isolated from the spinal fluid of patients presenting a clinical picture other than that of benign lymphocitic meningitis. There were 3 patients with encephalitis, 2 with meningoencephalitis and 1 who showed no evidence of encephalopathy. The virus was isolated as late as fifty-three days after the onset of acute symptoms, suggesting that the infection may be prolonged. The pathologic observations in 1 fatal case of encephalitis will be presented.

The strains isolated show some differences in their infectivity for different animal species, but all are immunologically related to the Rivers "W. E." strain, as shown by studies of reinoculated immune animals. Up to the time of writing, neutralizing antibodies have been demonstrated in only 1 patient, and those in low titer forty-five weeks after the onset of acute illness.

Anatomic Changes in Dogs Following Injections of Hematin. W. A. D. ANDERSON, E. F. WILLIAMS and D. B. MORRISON, University of Tennessee, Memphis.

Hematin in the form of disodium ferrihemate was injected into a series of dogs by subcutaneous, intravenous and intraperitoneal routes. At autopsy these dogs and their controls were examined by gross and microscopic methods. Widespread deposition of a brown pigment, which failed to give a prussian blue reaction, was found in mononuclear phagocytic cells and in cells of the reticuloendothelial system. Large amounts of the pigment were thus held within phagocytic cells and apparently remained innocuous. Vascular changes in the form of small hemorrhages and pigment thrombi occurred most freqently in the brain, endocardium and myocardium, particularly after intravenous administration. Striking lesions appeared in the kidneys. The most severe form was characterized by pronounced degenerative changes or massive necrosis of the convoluted tubules. Glomerular changes consisted of cellular proliferation, accumulation of polymorphonuclear leukocytes and formation of hyaline thrombi. Intraperitoneal admin-

istration of disodium ferrihemate over longer periods resulted in marked pigmentation of renal tubular cells and chronic changes in glomeruli. The changes produced by hematin are interesting in their similarity to the lesions of malignant tertian malaria and of hemoglobinuria from other causes.

Does Castration Alter the Incidence of Leukosis in Male Fowl? DAVID MARINE and SAMUEL H. ROSEN, Montefiore Hospital, New York.

Thirteen instances of leukosis have occurred in 119 attempted castrations in white Leghorns. The leukosis was the diffuse hepatorenal type in 8, lymphosarcoma in 4 and a mixture of types in 1. The birds were obtained at the age of 5 to 6 weeks, in five different batches, from three producers, and leukosis has developed in all batches. The youngest to die of the disease was 267 days old, and the oldest, 467. Fifty-three have been killed as slips. None of these slips showed any signs of leukosis, although of the same average age at death. None of the fowl with leukosis showed slitlike comb growth, although 2 had larger fragments of the left testis than some of the slips. All fowl with leukosis responded to androgens after they were known to have the disease. These data suggest that lack of testis hormones may have an activating effect on latent leukosis.

Production of the Thomsen Hemagglutination Phenomenon in Serum. I. DAVIDSOHN and B. TOHARSKY, Mount Sinai Hospital, Chicago.

From an O serum which was found to have the property of clumping O cells, a gram-positive bacillus was isolated which when inoculated into other serums, regardless of the blood group, causes those serums to agglutinate cells of all blood groups. Microscopically, it closely resembles members of the genus Corynebacteria.

When 52 individual serums, representing all the blood groups, and 10 pooled serums, each of the same blood group, were inoculated with the isolated bacillus, nonspecific agglutinins developed in 30 of the individual serums and in 9 of the pooled serums. The irregular agglutinins were demonstrated in undiluted serum and in diluted serum up to the dilution of 1:4. The agglutinins were active at 37 C. as well as at room temperature. On incubation at 37 C. and at 4 C., no change took place in serums inoculated with the bacterium.

Serums inoculated with the filtrate from a broth culture of the bacterium and incubated at room temperature contained nonspecific agglutinins at the end of four days. None incubated at 37 C. showed irregular agglutinins.

When the bacterium is inoculated into 2 per cent saline suspensions of cells of any blood group, it causes those cells to become panagglutinable by human serums. The same result is produced by the addition of proper amounts of a filtrate of a broth culture of the bacterium to suspensions of red blood cells.

Degeneration of Sensory Neurons in Pigs. MAXWELL M. WINTROBE, JOSEPH L. MILLER JR., HERMANN LISCO and LAWRENCE R. KOLB, Johns Hopkins Hospital, Baltimore.

With the purpose of producing a condition similar to pernicious anemia, pigs weaned at an early age (3 weeks) were given a diet consisting of casein (25.8 per cent), sucrose (56.9 per cent), lard (10.8 per cent), cod liver oil (1.3 per cent) and a salt mixture, supplemented with brewers' yeast (3 Gm. per kilogram of body weight).

When they became accustomed to this artificial diet and seemed to be in a good nutritive state, the quantity of yeast given some of the animals was gradually reduced and thiamin, riboflavin, nicotinic acid and filtrate factor were given instead, separately and in various combinations. Ataxia developed in *all* of these animals, in spite of good growth in those receiving nicotinic acid. There was

degeneration in the posterior columns of the spinal cord, in the posterior root ganglions and in the peripheral nerves of the affected animals, in various degrees.

In half the animals whose supply of yeast was not reduced, the ataxia and anatomic changes in the nervous system did not occur, and in the remainder the histologic changes were usually less extensive, although in several the ataxia seemed to be as great as in the pigs deprived of yeast. Wheat germ oil did not protect animals deprived of yeast. Observations to date indicate that a protective substance may be present in liver.

Effect of Loss of Gastric Juice on the Electrolyte Balance of the Dog. CHARLES C. MACMAHON and STEPHEN MADDOCK, Boston City Hospital.

Studies were made on dogs and pigs. A Heidenhain pouch was made in the fundic portion of the stomach, and the juice which was secreted was collected throughout each day. By returning this juice to the animals by stomach tube it was found possible to maintain them in an apparently normal condition for long periods. When the juice collected from the pouch was withdrawn and discarded, the well known characteristic clinical and chemical changes in the blood appeared. If the animals were given sodium chloride by mouth the gastric juice could be discarded without chemical changes occurring in the blood. When the sodium intake was kept up to normal by oral administration of sodium acetate, the animals remained in good clinical condition despite a fall in chloride ion to values as low as 65 milliequivalents per liter and a rise in carbon dioxide combining power to as high as 95 volumes per cent. With the sodium ion level maintained within normal limits (140 to 150 milliequivalents per liter), there was no significant elevation of nonprotein nitrogen or other signs of dehydration (oliguria, hemoconcentration, anorexia, weakness) despite the marked hypochloremia and alkalosis.

The maintenance of a normal sodium balance became difficult when the chloride levels fell below 60 milliequivalents per liter, and at levels as low as 40 to 45 milliequivalents per liter became impossible. Then the characteristic train of chemical and clinical changes developed. In animals deprived of the sodium as well as of the chloride ion these changes appeared when the chloride level approached 80 milliequivalents per liter. This relationship of rise in nonprotein nitrogen to fall in sodium ion in the electrolyte balance emphasizes the importance of the basic ion in maintaining a state of normal hydration.

Effect of Complete and of Partial Biliary Obstruction on the Serum Phosphatase and Bile Acids of the Dog. STEPHEN MADDOCK, S. J. THANNHAUSER, VICTORIA MAGHEE and HAROLD REINSTEIN, Boston City Hospital and Boston Dispensary.

We have stated previously that the ligation of one or more of the four hepatic ducts of the dog produced a transient rise in serum phosphatase, although jaundice did not supervene. Schiffman and Winkleman reported later that ligation of a single hepatic duct in each of 2 dogs led to an increase in phosphatase which persisted for thirty days. These authors apparently concluded that this was an invariable result. Our work has shown that some dogs fail to show such a rise following ligation of one hepatic duct, but that with ligation of two hepatic ducts all present a rise, which may be persistent. The ligation of the cystic duct can also produce marked elevation of serum phosphatase.

The serum phosphatase values in complete biliary obstruction in 40 dogs showed great variation in individual animals. In some the values did not rise much above 100 Bodansky units but in others the values rose as high as 460 Bodansky units eight weeks after ligation. Our experience has been that the values rise in a fairly smooth curve and gradually decline up to the time of death. This variation in the individual reactions of the animals led us to conclude that transfusion of normal blood to a jaundiced dog causes a rise in serum phosphatase. We now feel that such a statement must be made with some reservations until further work can be done.

In some of the animals bile acids have been studied by a recently published method of Jenke. This method has the advantage of giving a zero value for the blood of a normal animal. Following the ligation of the common duct there is a sharp rise in blood bile acids with a gradual decline after three or more weeks.

Response of Different Strains of Rats to Nephrotoxin. JOSEPH E. SMADEL and HOMER F. SWIFT, Hospital of Rockefeller Institute for Medical Research, New York.

Earlier studies on rats of the Whelan strain, in which severe nephritis had been induced with anti-rat-kidney serum, showed that in nephritic animals fed a high protein diet consistently a progressive disease developed from which they died within four to twelve months, whereas nephritic animals fed a low protein diet tended to recover from their acute nephritis. Administration of comparable amounts of nephrotoxin to rats of the Whelan and Evans strains resulted in acute renal injury of about equal severity. Wistar rats, on the other hand, displayed a milder degree of acute injury following the same dose of nephrotoxin. The acute nephritis regressed rapidly during the first month in Evans and Wistar rats maintained on a high protein diet. In the majority of these animals, however, recurrences developed after a latent period of two to six months. Although their urine became quite abnormal, only a few of these rats died of renal failure during the fourteen months of observation.

Differences in the response of the three strains of rats were dependent on hereditary factors and not on strain specificity of the nephrotoxin, since nephrotoxin prepared in rabbits by injecting kidney tissue from Whelan and from Wistar rats induced similar renal reactions.

Production of Pressor Substance by Anaerobic Autolysis of Renal Cortex. JOSEPH VICTOR, ALFRED STEINER and DAVID M. WEEKS, New York City Hospital and Columbia University, New York.

The experiments to be described were concerned with the role of oxygen in the elaboration of pressor substance by the kidney. Dog renal cortex was sliced about 0.2 mm. thick and incubated in 2 parts of plasma or Krebs solution at 37.5 C. in the presence and in the absence of oxygen for one to three days. The fluid filtered from the mixtures autolyzed in nitrogen showed on intravenous administration a powerful pressor action. The systolic blood pressure increased 20 to 300 mm. of mercury in 24 tests in 7 dogs. The fluid of the mixtures autolyzed in oxygen, on the contrary, decreased the blood pressure 40 to 186 mm. in 7 of 10 tests in 5 dogs. When the medulla of the kidney was prepared in a similar fashion, the fluid of the mixtures autolyzed in nitrogen increased the blood pressure only 4 to 25 mm. in 5 of 7 tests in 6 dogs. Medulla incubated in oxygen decreased blood pressure in 2 of 3 dogs.

Similar results occurred when sterile, cell-free saline extracts of beef or dog renal cortex or medulla were treated in the manner described. No pressor effects were obtained by comparable treatment of sterile, cell-free extracts of liver, spleen, lung or heart muscle.

Effect of Induced Collateral Circulation from the Spleen to the Ischemic Kidney on Experimental Renal Hypertension. DAVID M. WEEKS, ALFRED STEINER, JAMES S. MANSFIELD and JOSEPH VICTOR, New York City Hospital and Columbia University, New York.

The object of this investigation was to study (1) the influence on blood pressure of pexis of the spleen to the ischemic kidney, (2) the existence of collateral circulation in such preparations and (3) the effect on blood pressure of the subsequent removal of the joined organs. After a group of 8 dogs was maintained hypertensive by the Goldblatt method, an operation joining the cut parenchymas of spleen and kidney was performed on each. The blood pressure of all 8 dogs fell following this

operation. Of the 7 animals showing a persistent fall in blood pressure, collateral circulation to the capillaries about the tubules, and not in the glomeruli, was demonstrated in 5 by injections of india ink and microscopic examination. In the animals surviving the removal of the pexis more than three days, an elevation of blood pressure to the previous hypertensive level occurred. A collateral circulation established by pexis of spleen and ischemic kidney increases the blood supply to the renal tubules and lowers the blood pressure in experimental hypertensive dogs.

Effect of Nitrogen Retention on the Regeneration of Plasma Proteins. RUSSELL L. HOLMAN and J. GILMER MEBANE, University of North Carolina, Chapel Hill.

Experiments were performed to determine whether the impaired formation of plasma proteins in nephritis is related to retention of nitrogen or to other factors. In normal adult dogs, maintained on a standard low protein diet, the plasma proteins, including the reserve stores were depleted to a basal level of 4 per cent by repeated bleeding and return of the washed red blood cells suspended in saline solution (plasmapheresis). After the hypoproteinemic state had been established, uranium nitrate was injected subcutaneously, and the effect of the ensuing elevation in nonprotein nitrogen on the production of plasma proteins was followed. Three dogs have been tried, with uniformly negative results. The data will be presented and their significance discussed.

The Antianemic Principle in Liver from Patients with Carcinoma of the Stomach and of the Cecum. JOHN R. SCHENKEN, JOSEPH STASNEY and W. KNOWLTON HALL, Louisiana State University and Charity Hospital of Louisiana, New Orleans.

The administration of an extract prepared from the liver of a patient whose death was due to a scirrhous carcinoma of the pyloric and prepyloric portions of the stomach to 2 patients with typical addisonian pernicious anemia failed to produce a reticulocyte response. The administration of a control extract prepared under exactly the same circumstances from the liver of a patient whose death was due to cerebral hemorrhage caused marked hemopoiesis in a patient with typical pernicious anemia.

Extracts prepared from the livers of 2 patients who had died, the first with an extensive carcinoma of the stomach without involvement of the pylorus and the second with carcinoma of the cecum, stimulated hemopoiesis when injected into patients with macrocytic hyperchromic anemia.

The antianemic principle in the liver was absent in the patient whose pyloric mucosa was replaced by neoplastic tissue and present in the liver of the patient whose entire stomach except the pylorus was involved by carcinoma. It is of interest that the mucosa of the pyloric region, according to experimental observations, is the most active in the production of the "intrinsic factor."

Effect of Varying Doses of Estrogen on the Incidence of Mammary Gland Carcinoma in Strain C_3H Mice. JOHN R. SCHENKEN and EDWARD L. BURNS, Louisiana State University, New Orleans.

The relationship between carcinoma of the mammary gland and estrogen was tested quantitatively by injecting progynon B into 122 male mice of strain C_3H, beginning at the age of 2 weeks. These animals were divided into seven groups: Group 1 received 3,000 rat units in two doses over a period of three days. The remaining six groups received 100 rat units weekly for varying periods of time as follows: group 2, four weeks; group 3, eight weeks; group 4, twelve weeks; group 5, sixteen weeks; group 6, twenty weeks; group 7, continuously throughout the lifetimes of the animals. The controls were 28 untreated breeding males

and 46 untreated breeding females of strain C$_3$H. Only animals which had lived five months or longer were included in this study. The incidence of tumors of the mammary glands was determined at necropsy.

No tumors developed in animals of groups 1 or 2. A low incidence of tumors was observed in animals of groups 3 and 4. A relatively high and essentially equal occurrence of tumors was noted in mice of groups 5, 6 and 7. No tumors were noted among the male controls. A slightly higher percentage of the female controls showed tumors than of any of the experimental groups.

Effect of Intracutaneous Growth of Brown-Pearce Tumor on Preexisting Testicular Tumor. O. SAPHIR, M. APPEL and A. A. STRAUSS, Michael Reese Hospital, Chicago.

It is well known that the transplantation of Brown-Peace carcinoma into the testicle of a rabbit results in a large tumor with diffuse metastases. Spontaneous regression is rare. It is also recognized that the intracutaneous transplantation of Brown-Pearce carcinoma in a rabbit results in a cutaneous tumor which soon regresses, leaving the animal immune to subsequent transplantation of this tumor, regardless of the site of transplantation. These experiments brought forward an additional fact, namely, that in rabbits already afflicted with testicular Brown-Pearce carcinoma and metastases the intracutaneous transplantation of homologous tumor results in cutaneous tumors which often regress. Furthermore, following regression, the preexisting and coexisting testicular tumors and their metastases also undergo regression. The histologic features of these regressing tumors are discussed.

Distribution of Large Doses of Radioactive Phosphorus in Rats. SHIELDS WARREN and R. F. COWING, Harvard University, Boston.

Radioactive phosphorus, prepared by the department of physics of Harvard University by neutron bombardment of red phosphorus, was injected, in the form of dibasic sodium phosphate, intraperitoneally in rats. The dose given ranged from 16 to 30 microcuries per animal. The content in the blood dropped in the first forty-eight hours, and the content in the urine fell sharply also. Approximately 6 per cent of the amount injected was excreted in the first three hours. Measurements were made with a Lauritsen type electroscope. The experimental error is probably 15 per cent. The tissues containing most phosphorus were: liver, voluntary muscle, bone, spleen and kidneys. The histologic observations will be reported.

Induced Tumors of Salivary Glands of Mice. HAROLD L. STEWART, National Cancer Institute, Bethesda, Md.

A solution of 1,2,5,6-dibenzanthracene or methylcholanthrene in lard or light liquid petrolatum was injected into the salivary glands of 80 male and female mice of strains A and C$_3$H. In 12 additional male mice of strain A, cotton threads coated with crystalline benzpyrene were stitched into the glands, one in each, and allowed to remain. Tumors developed in 69 mice. Histologically, the tumors were squamous cell or adenosquamous cell carcinoma, spindle cell sarcoma, rhabdomyosarcoma or a mixed growth composed of one or more of the foregoing types. One tumor was unclassifiable. The average latent period for the development of the tumors in 19 male strain C$_3$H mice and in 26 male strain A mice in which 0.34 mg. of methylcholanthrene had been injected into the salivary glands was thirteen and one-tenth weeks and twelve and seven-tenths weeks, respectively. The possibility that the sarcomas may have originated from the muscular and connective tissues surrounding the salivary glands is under investigation.

Radioactive Iodine as an Indicator in Thyroid Physiology: Observations on Patients with Goiter. SAUL HERTZ, A. ROBERTS, J. H. MEANS and R. D. EVANS, Massachusetts General Hospital, Boston.

In a series of patients with goiters of various types it was found possible to trace iodine by means of radioactive "labeling" and to determine not only the uptake by the gland but the rate of excretion of the iodine from the body. As a result, certain inferences as to the possibility of therapeutic application of radioactive iodine can now be made.

Coronary and Aortic Sclerosis, Periarteritis Nodosa, Chronic Nephritis and Hypertension as Sequelae to a Single Experimentally Produced Widespread Calcium Precipitation in the Rat. ARTHUR W. HAM, University of Toronto, Ontario, Canada.

Rats fed either (1) a single massive dose of activated ergosterol or (2) a diet rich in calcium, phosphorus and phosphoric acid for three weeks were found to have, soon after, widespread calcification of their vascular systems and kidneys. The initial calcification in the arteries was followed in both cases by intimal proliferation, which seemed to be of a reparative character. Several series of animals given a massive dose of activated ergosterol were allowed to live until they died of the complications arising from the original calcification. These animals seldom lived for a year, but most of them lived for more than six months. At autopsy they presented a picture of severe arterial and renal disease, complicated by hypertension. The original lesion in the kidney consisted of calcification of tubules in the loop of Henle, calcification in and about the glomerular tufts and calcification of glomerular arterioles, as well as calcification in some of the larger vessels. This original lesion was followed by glomerular fibrosis and cyst formation in blocked tubules. Hyalinization in glomeruli and in arteries became prominent later, as did periarteritis nodosa in the intestinal arteries. More than half the animals revealed the latter condition, which has been shown by others to be related to hypertension in the rat. None of the controls exhibited this lesion.

Transformation of the Mycelial Form of Histoplasma Capsulatum, Darling, to the Yeastlike Form in Mice. ROBERT J. PARSONS, University of Michigan, Ann Arbor.

Recently Histoplasma capsulatum was isolated on biopsy of a nasal septal ulcer of a female patient. The granulomatous tissue about the ulcer contained great numbers of large mononuclear phagocytes which were heavily parasitized by yeastlike bodies, tentatively designated as Histoplasma capsulatum. Cultures of the ulcer base on tartaric acid–agar medium at room temperatures (Pellett) resulted in growth of the mycelial form of H. capsulatum. Since only the yeastlike form produces progressive disease in man and animals, I wished, for experimental purposes, to transform the mycelial to the yeastlike form. This was successfully accomplished by injecting the mycelial form intravenously into young mice. Autopsy of the mice has shown extensive parasitization of the so-called macrophage system of cells by the yeastlike form of the fungus.

Genesis of Primary Pulmonary Lesions in Experimental Tuberculosis of Dogs. F. D. GUNN and MOORE A. MILLS, Northwestern University, Chicago.

The result of intrabronchial insufflation of adequate doses of virulent tubercle bacilli (human or bovine type) in dogs is at first a localized, low grade inflammatory reaction in the parenchyma, characterized by exudation of polymorphonuclear leukocytes and monocytes and by proliferation of septal cells. Cells of the large mononuclear exudate type predominate after about twenty-four hours. The lesions enlarge by direct extension in all directions and become solid in the center, and

necrosis begins in one or more foci, usually in several places at the same time in the larger pneumonic lesions. After caseation has become extensive, evacuation of necrotic material may occur if a bronchus of sufficient magnitude is involved. In this case, reaspiration of infectious material results in the formation of numerous foci of tuberculous bronchopneumonia in various parts of the lung. Otherwise, the mass becomes slowly encapsulated, calcium salts are deposited in the caseous matter, and after several months stainable acid-fast bacilli may disappear from the lesion.

Encapsulation of a lesion does not necessarily mean complete arrest of the inflammatory process, since the capsule tends to form just outside of the necrotic center and does not surround the entire granulomatous lesion.

Regional lymph nodes are involved within a few days after initiation of the parenchymal lesion. The degree of histiocytic hyperplasia and necrosis of lymphoid tissue depends on the number of bacilli reaching the node. Caseation of lymph nodes usually occurs in the presence of caseous parenchymal lesions. Calcification occurs after a few or several months in a small percentage of cases.

A New Approach in the Therapy of Septicemia Due to Streptococcus Viridans in Experimental Animals. O. M. GRUHZIT, Parke, Davis and Company, Detroit.

A study of substances of the sulfanilamide type in which the sulfur group had been substituted with carbon, oxygen, nitrogen, phosphorus, arsenic or their oxidation products resulted in the discovery of substances active against Streptococcus viridans in mice. Of these the paranitromethylbenzene and its oxidation product, the paranitrobenzoate, possessed marked effect; doses as low as 0.15 mg. per mouse administered orally twice daily for three days prevented death in 80 per cent of mice whereas sulfanilamide gave negative results and sulfapyridine showed only slight activity. The paranitrobenzoate exerts a specific effect on Str. viridans in mice similar to that of sulfanilamide on the betahemolytic streptococcus. It possesses only slight activity on betahemolytic streptococci in mice and shows no activity against type I pneumococci or in tuberculous guinea pigs. The paranitrobenzoate is relatively nontoxic. Orally, for mice the minimal lethal dose is about 30 mg. per mouse, and for rats it is about 2.5 Gm. per kilogram; intravenously, the minimal lethal dose for rats is about 1.3 Gm. per kilogram. Dogs receiving daily 50 to 200 mg. per kilogram for two or three weeks have not shown anemia or cyanosis. Dogs have not shown nausea, vomiting or incoordination with doses of 300 mg. per kilogram. Pigeons receiving 1.0 Gm. per kilogram for seven days remained free from cyanosis or other complications. Experimentally, the administration of paranitrobenzoate appears to be a specific therapy for Str. viridans infection in mice.

Influence of Diet on the Survival of Rats Repeatedly Exposed to Carbon Tetrachloride Vapor. JESSE L. BOLLMAN, The Mayo Clinic, Rochester, Minn.

Rats fed a diet adequate for moderate growth, which contained 40 per cent animal protein, 40 per cent carbohydrate and 8 per cent fat, were subjected to carbon tetrachloride vapor for thirty minutes three times each week. The average survival time of this group was thirty-one and three-tenths days. Other rats were fed one half of the control diet, the other half being replaced by an isocaloric equivalent of carbohydrate, protein or fat, respectively. Considering the survival time of the rats fed the control diet as 100 per cent, the survival time of the animals fed carbohydrate, protein or fat was 156 per cent, 105 per cent and 87 per cent, respectively. None of the treated animals consumed as much food as the untreated animals. The ratio of the food consumption was control diet 72, carbohydrate 82, protein 86 and fat 71. The animals of each group which consumed more than the average amount of food, however, did not survive longer than the other members of the group.

Marked necrosis of the liver was produced in all of the treated animals, and cirrhosis was extensive in all that survived a few weeks. Most of the animals died with massive hemorrhage into the small intestine. Such hemorrhages were not prevented by the administration of vitamin K by mouth. The histologic picture of the intestinal hemorrhages will be demonstrated.

The Agent of Lymphogranuloma Venereum in the Fertile Hen's Egg. GEOFFREY RAKE, CLARA M. MCKEE and MORRIS F. SHAFFER, Squibb Institute for Medical Research, New Brunswick, N. J.

In an investigation of the agent of lymphogranuloma venereum, with a view to studying the activity of certain chemotherapeutic drugs thereon, an initial difficulty was encountered in the low pathogenicity for experimental animals of the strains of lymphogranuloma venereum available. Use of Burnet's inoculation method in the fertile hen's egg did not produce much more satisfactory results. When, however, the yolk sac method of inoculation suggested by Cox was adopted, the virus was found to multiply very readily in the walls of this sac. Over twenty-five passages have been made by this method. Both infectivity and lethal titers of the agent for the developing chick through 10^{-9} have been obtained. Centrifugation of yolk sac preparations first at 3,000 and later at 10,000 revolutions per minute gives a sediment of small granules resembling morphologically the elementary bodies of the virus of vaccinia and similar to the granules described as specific for the agent of lymphogranuloma venereum by other workers. These granules appear to represent the infectious agent. They show a high infectivity compared to the supernatant obtained by supercentrifugation. The granules evoke specific antibodies in rabbits and chickens.

Cultivation of the Virus of Lymphogranuloma Venereum on the Chorioallantoic Membrane. MARION E. HOWARD, Yale University, New Haven, Conn.

The virus of lymphogranuloma venereum can be propagated on the chorioallantoic membrane of the developing egg. Specific lesions were observed in only 30 per cent of the membranes, usually in those incubated four to six days. Histologically, these lesions resembled the small abscesses found early in infected human glands. Success of propagation is dependent on the time of transfer for a particular strain and the temperature of inoculation.

Egg membranes made into Frei antigen and tested in known cases elicited skin reactions varying in size. This variation seemed to be dependent on the presence or absence of lesions as well as on the time of incubation. Membranes which had been incubated from four to six days and which showed lesions usually gave the largest skin reactions. The infectivity of membranes seemed greatest after two to three days of incubation and was greatly reduced or absent after four to six days. Infectivity and antigenicity do not run parallel. This may explain some of the variations in human antigens.

Effect of Extravasated Antibody on the Antigenicity of Extracts of Virus-Induced Rabbit Papillomas. JOHN G. KIDD, Rockefeller Institute for Medical Research, New York.

The results of experiments will be given which show that large quantities of the rabbit papilloma virus are rendered completely nonantigenic on neutralization in vitro with the antiviral antibody and that extracts of the virus-induced growths of wild and domestic rabbits which contain much extravasated antibody may fail to stimulate the production of antibody even when massive amounts are injected repeatedly into normal rabbits. The fact was discovered incidentally that passively transferred antibody may be responsible for resistance to the papilloma virus following injections of extracts of the growths intraperitoneally into normal rabbits.

The findings disclose the strict limitations of immunization experiments of the sort described as a means of demonstrating whether "masked" virus is or is not present in extracts of the virus-induced growths. Since extravasated antibody is usually present in the growths, and since it not only neutralizes the virus when the growths are extracted but also renders the extract nonantigenic, it appears to be impossible to demonstrate by this method that "masked" virus is absent from a given extract unless it is known that antibody is also absent. The cancers deriving from the virus-induced papillomas of cottontail rabbits frequently contain an excess of extravasated antibody, and hence it follows that the attempted immunization of normal rabbits with extracts of these growths would fail to provide decisive evidence as to whether the virus is or is not present in them.

Lesions of the Tissues in Dehydration Shock. HARRY A. DAVIS, Louisiana State University, New Orleans.

Dehydration shock was produced in dogs by injecting subcutaneously 25 per cent sodium chloride solution in doses of 25 cc. per kilogram of body weight. All of the animals presented evidences of extreme hemoconcentration, and death occurred within five to fifteen hours. The brain was cyanotic and revealed petechial hemorrhages in the leptomeninx and underlying cortical tissue. The capillaries were extremely distended, and the number of those visible was increased by actual count.

The lungs were reddish blue and studded with minute and larger hemorrhages. The capillaries in the alveolar walls were packed with red blood cells, and there was extravasation into the alveoli and beneath the visceral pleura. In those animals which survived for ten hours or more there were, in addition, focal areas of pulmonary edema. There were subendocardial and subepicardial hemorrhages, with petechiae between the myocardial fibers. The capillaries of the serosae and of the mucosae of the stomach, duodenum, jejunum and rectum were distended, so that these surfaces appeared dark red. The spleen revealed marked distention of the sinusoids and extensive hemorrhages beneath the capsule and about the malpighian corpuscles. The capillaries of the zona fasciculata and zona reticularis of the adrenal glands were markedly distended. In addition, there were petechial hemorrhages with minute areas of necrosis and diffuse and focal collections of polymorphonuclear leukocytes in this portion of the adrenal cortex. In the liver there was extreme distention of the capillaries about the central veins, as well as hemorrhages into the liver substance and beneath the capsule. The capillaries of the kidneys were distended, and there was marked albuminous change in the epithelial cells of the convoluted tubules. There was edema of the subcutaneous and intermuscular connective tissue at the site of injection, but no necrosis.

Chemical Analysis of Vaccinal Elementary Bodies. CHARLES L. HOAGLAND, JOSEPH E. SMADEL and THOMAS M. RIVERS, Rockefeller Institute for Medical Research, New York.

A chemical investigation of relatively pure preparations of elementary bodies of vaccinia, begun by Hughes, Parker and Rivers in 1935, has been extended to include quantitative determination of cholesterol, cholesterol esters, phospholipids, neutral fats, reducing sugars, amino acids, nitrogen, carbon and phosphorus. The analyses have also included similar determinations on the crude dermal pulp and on various discarded sediments from which the elementary bodies were finally separated. Whereas materials discarded early in the course of purification have been found to vary markedly in their chemical makeup from lot to lot (total fat, 8.0 to 10.0 per cent; nitrogen, 11.5 to 13.8 per cent; phosphorus, 0.3 to 0.48 per cent; reducing sugars after hydrolysis, 1.9 to 2.3 per cent), the final preparations of elementary bodies show a surprising constancy of analytic values (total fat, 4.0 to 5.5 per cent; nitrogen, 14.9 to 15.3 per cent; phosphorus, 0.57 to 0.59 per cent; reducing sugars, 2.5 to 3.0 per cent). Moreover, intradermal titrations of

each lot revealed a high degree of infectivity, consistent with the constancy of the analytic data. Such data tend to show a degree of chemical complexity not observed in certain plant viruses but approaching that of more highly organized bacteria and protozoa.

Specific Neutralization of the Virus of Myxoma. ROBERT F. PARKER, Western Reserve University, Cleveland.

Reliable results in the titration of the neutralizing potency of immune serum can be obtained when the "50 per cent" end point is used in titrating the virus. This end point, a theoretic dilution of virus which on inoculation should give rise to 50 per cent positive results, is calculated from the results of quadruplicate inoculation of tenfold dilutions of virus. The use of this or of a similar end point is necessitated by the particulate nature of the virus. Neutralizing capacity is then defined as the difference in titer of virus when it is titrated in the presence of normal and of a given dilution of immune serum.

By this test it has been found that serum of animals convalescent from infection with myxoma neutralizes small but constant amounts of virus and that the amount neutralized bears a regular relation to the concentration of serum. Serum of animals given injections of solutions of soluble specific substances but not active virus also neutralizes appreciable amounts of virus.

Further Inoculation Experiments with the Virus of the Common Cold. H. M. POWELL, A. L. SPARKS and G. H. A. CLOWES, Eli Lilly and Company, Indianapolis.

Six strains of the virus of the common cold following tissue culture propagation have been passed through Swiss mice, with scarcely more than very mild lesions developing. Virus alone did not kill; a few deaths occurred when organisms resembling Pfeiffer's bacillus were encountered as associated bacteria. Several passages of the virus through ferrets have been made, and frequent responses of temperature have been observed. We have observed little local effect when either attenuated or raw virus has been injected into human subjects. Raw virus when used in human subjects has appeared now and then to have been the cause of colds, so that further work is necessary before this agent can be used in man with success.

Effect of Immune Serum and Vaccines on Experimental Arthritis Produced by the Filtrable Micro-Organisms of the Mouse Pleuropneumonia Group. ALBERT B. SABIN and ISABEL M. MORGAN, Rockefeller Institute for Medical Research, New York.

The filtrable micro-organisms of the mouse pleuropneumonia group can be classified neither with bacteria nor with viruses. Since their pathogenic properties appear to depend on their invasion of and intracytoplasmic multiplication in certain mesenchymal cells, it was of interest to determine the effect of immune serum and vaccines before and after infection. Type B cultures intravenously injected into mice produced progressive, proliferative polyarthritis, with no evidence of involvement of any other organs or tissues. One cubic centimeter of immune rabbit serum (agglutinin titer, 1:2,000) injected intraperitoneally before injection of a culture completely prevented the arthritis in practically all mice; normal rabbit serum was without effect. When the immune serum was withheld until definite arthritis appeared, there was no obvious effect for a week or two; then the joints which had already become involved continued to become more severely diseased and even other joints became involved; after that time the arthritis in about 50 per cent of the mice cleared, while in the others it continued (in some to ankylosis) as in the controls. Vaccination with concentrated suspensions of micro-organisms killed by heating at 50 C. for one-half hour did not have as good an effect as the

administration of immune serum, but qualitatively the results were of the same order. The type A micro-organism produces a different disease, which was affected by homologous immune serum in a similar manner.

Role of Natural and of Acquired Immunity in Recovery from Virus Infections. ROBERT G. GREEN, University of Minnesota, Minneapolis.

In some virus diseases recovery may depend more on the natural resistance of the host at the time of onset than on the acquired immunity arising during the course of the infection. The natural immunity seems to be due to a resistance of cells to invasion by the virus as well as to the presence of antivirus in the blood. The degree of susceptibility of the host is determined largely by the degree of susceptibility of those systems of cells to which the virus is adapted. In fox encephalitis recovery from the natural disease appears to be entirely dependent on the degree of natural immunity at the time of onset since fatalities occur regularly within the first five days of the disease and acquired immunity develops slowly. The injection of combinations of antiserum and live virus as vaccines results in some fatalities, which may be either early or delayed but are principally the latter. A slight degree of immunity acquired from a killed virus vaccine will protect against fatalities from serum and live virus combinations.

Production of Immunity in Ferrets Against Various Strains of the Virus of Epidemic Influenza. FRANK L. HORSFALL JR. and EDWIN H. LENNETTE, the Laboratories of the International Health Division of the Rockefeller Foundation, New York.

A vaccine containing both ferret spleen and lung has been found to be effective in producing immunity against different strains of the virus of epidemic influenza. The vaccine was prepared from tissues obtained from ferrets eleven days after intranasal inoculation of a 1939 strain of the virus and was inactivated by formaldehyde. A single subcutaneous injection of the vaccine resulted in rapid development of solid immunity against infection by strains of quite different antigenic structures. Vaccinated ferrets were found to be immune to both the PR8 and the W. S. strains as well as to various 1939 strains of the virus. The serum of vaccinated ferrets possessed the capacity of neutralizing large amounts of different strains of the virus within a few days after vaccination and thereafter.

Vaccines prepared in an identical manner from the tissues of ferrets which had been inoculated with other strains of the virus have failed consistently to produce immunity after subcutaneous inoculation.

Book Reviews

Pathology: An Introduction to Medicine and Surgery. J. Henry Dible, Professor of Pathology in the University of London, and Thomas B. Davie, Professor of Pathology in the University of Liverpool. Pp. 931, with 374 illustrations, including 8 plates in color. Price, $10. Philadelphia: The Blakiston Company, 1940.

The authors of this volume are experienced teachers of pathology with well defined views on methods of teaching. The book is based on lectures which they have delivered to medical students. This is evident from the agreeable informality, without loss of dignity or seriousness, that characterizes their presentation. Pathologic changes are considered as a series of processes going on in the living body and leading to certain consequences—signs, symptoms, functional changes and morbid anatomic results. In the text greater effort has been made to teach how the diseased conditions arise than how to recognize them once the morbid anatomic changes have become established. The authors insist that the right places to learn to recognize morbid anatomic conditions are the postmortem room and the museum and that no amount of textbook teaching can supplant these. This attitude explains some of the conspicuous features of the book.

The conventional subdivisions, general pathology and special pathology, have not been followed. As to the arrangement of their material the authors state that they have "fallen back upon that characteristically English solution of a difficulty: a compromise." Inflammation, as the most fundamental process in a pathologic involvement, is considered first and then follow chapters on circulatory disturbances, degenerations, tumors and immunity. This constitutes the first section of the book, covering 234 pages, with 119 illustrations. The discussion of general principles and that of tumors (to which 79 pages are devoted) are briefer than is usually considered adequate for medical students in this country. This brevity is made possible by emphasizing the dynamics of the various processes and by giving less attention to the more static features of gross and microscopic appearances.

The second section of the book, 165 pages, deals with special infections. This section begins with a detailed discussion of three illustrative types—of anthrax, as an example of a septicemic disease; of diphtheria, as a toxemia, and of actinomycosis, as a subacute inflammatory process. This is followed by briefer consideration of common infections, classified according to their causative agents—staphylococcic, streptococcic, mycobacterial, spirillar and anaerobic infections, rheumatism, gonorrhea, undulant fever, glanders, cholera and plague, and virus diseases (a chapter).

The third and final section, 397 pages, is given up to systemic diseases, and in it the more important pathologic conditions which have not already been dealt with are discussed. Animal parasitic diseases are omitted entirely; likewise, gynecologic conditions. In this third section a chapter on iron and pigment metabolism is placed between the chapters on the alimentary system and those on the genitourinary system.

Certain features of this volume deserve special mention. The value of the descriptions of the "technique of blood examination," pages 464-467, and of "tests for renal function," pages 767-772, in a book of this kind is questionable because their necessary brevity renders them inadequate. On the other hand, praise is due for the chapter on "Pathologic Changes in Respiratory Function" with special emphasis on Cheyne-Stokes respiration, hyperpnea, anoxemia and cyanosis; for the chapter on "Renal Function in Disease," i. e., the various types of nephritis, and for the table of "Findings in the Cerebrospinal Fluid." Among the numerous

tables and diagrams, most of which are pertinent and helpful, only a few can be mentioned. Figure 89 is a diagram of the spread of cancer from the primary growth by lymphatic permeation and embolism. In the diagrammatic figure 224 arrows indicate the direction of impedance to blood flow in cardiac decompensation due to left-sided valvular disease. Figure 276 illustrates the effect of emphysema of the chest on diaphragmatic respiration. Figure 305 shows the anastomoses between the portal and the systemic circulation. Figure 308 is a diagram of the production and relationship of the bile pigments. The schematic representation in figure 314, of the course and relationship of the different forms of nephritis, will be helpful. The effort to emphasize the clinical aspects of pathology is indicated by the inclusion of discussions of the chemotherapy of syphilis and of the serum treatment of pneumonia.

The subtitle, "An Introduction to Medicine and Surgery," reveals the purpose of the authors in preparing this book. It is doubtful if it will meet the requirements of American teachers of pathology as a textbook for medical students. But pathologists interested in teaching will find in it many useful methods of presenting material that is not new. The authors have made an effort, admittedly successful, to present the important facts of pathology from a dynamic rather than from a static point of view. To accomplish this they have omitted much that is considered indispensable in a standard textbook of pathology, but they have included much that usually finds no place in such a text. There has been a definite trend in this direction in the revised editions of textbooks on pathology during the past few years, but the present authors have gone beyond the writers of any of the more popular American texts. Because of the effective correlation between pathology and clinical medicine and surgery, this volume should be especially interesting and useful to practicing physicians. It will also meet adequately and effectively the requirements of a textbook in pathology for dental students because of its lack of unnecessary detail without undesirable abridgment of the descriptions of the more important pathologic processes.

In the selection of the 374 figures in the text the authors have intentionally "omitted many of those which illustrate common conditions which every student should be familiar with in the post-mortem room or the operating theater." The publishers have produced a book of excellent appearance. The type is large and easily readable, but with less important topics in smaller print. The illustrations are well reproduced, and their details are clear. Most of them leave little to be desired. The index occupies 34 pages and is well arranged, with the chief subjects announced in heavy type.

The Rise of Embryology. Arthur William Meyer, Professor of Anatomy, Emeritus, Stanford University. Cloth. Pp. 367, with 97 illustrations. Price, $6. Stanford University, Calif.: Stanford University Press, 1939.

In this volume Professor Meyer's historical talent finds its most ambitious expression. Some aspects of the subject matter have already been treated by him in independent essays, but most of the material is new. The method of approach is that of quotation from original sources, woven into a framework of explanation and comment. It is this presentation of obscure and widely scattered source material that makes the book especially valuable to readers who have neither the ability nor the patience to attempt the searching out, translating and collating of the original articles. Indeed, were ability and ambition equal to the task, access to the writings of many pioneers in biology would still be lacking to most.

Fifteen chapters treat of the following topics: early ideas of reproduction and generation; epigenesis; preformation; pangenesis; panspermia; search for the mammalian ovum; discovery, origin and meaning of the spermaozoon; changing ideas of impregnation; role of the "mule"; problem of malformation; early visual aids; growth of morphology; early experimental embryology. There are 97 illustrations, 408 entries in the bibliography and an adequate index.

The serious worker in biology and medicine will welcome Professor Meyer's contribution with gratitude. It represents a laborious task done well by one eminently competent to select judiciously, to interpret penetratingly and to weigh with rare discrimination. Even immature students in these sciences should be directed to such representative chapters as that on the changing ideas of impregnation and that on the search for the mammalian ovum. Only in this way can the tyro be made to "realize that it required long and arduous effort, and clear thinking as well, to establish even the simplest facts—those he now takes for granted."

Books Received

CLINICAL ROENTGENOLOGY OF THE ALIMENTARY TRACT. Jacob Buckstein, M.D., Visiting Roentgenologist (Alimentary Tract Division), Bellevue Hospital, New York; Consultant in Gastroenterology, Central Islip Hospital. Cloth. Pp. 652, with 525 illustrations. Price $10. Philadelphia and London: W. B. Saunders Company, 1940.

SHOCK AND RELATED CAPILLARY PHENOMENA. Virgil H. Moon, A.B., M.Sc., M.D., Professor of Pathology, Jefferson Medical College; Director of Laboratories, Jefferson Medical College Hospital; Visiting Chief Pathologist, Philadelphia General Hospital. Cloth. Pp. 442, with 30 illustrations. Price $3.50. London, New York and Toronto: Oxford University Press, 1938.

DERMATOLOGIC ALLERGY. AN INTRODUCTION IN THE FORM OF A SERIES OF LECTURES. Marion B. Sulzberger, M.D., Assistant Clinical Professor of Dermatology and Syphilology, Skin and Cancer Unit of the New York Post-Graduate Medical School and Hospital of Columbia University; Associate Attending in Dermatology and Syphilology, Montefiore Hospital, New York; Consultant in Dermatology and Syphilology, French Hospital, New York. Cloth. Pp. 540, with 48 illustrations. Price $8.50. Springfield, Ill., and Baltimore, Md.: Charles C. Thomas, Publisher, 1940.

DIRECTORY OF MEDICAL SPECIALISTS CERTIFIED BY AMERICAN BOARDS. 1939. Paul Titus, M.D., Directing Editor. Cloth. Pp. 1573. Price $5. New York: Columbia University Press, 1940.

MEDDELELSER FRA DR. F. G. GADES PATHOLOGISK-ANATOMISKE LABORATORIUM I BERGEN, 1939. Paper. Various pagination. Bergen, 1940.

LA CREATINE. ETUDE PHYSIO-PATHOLOGIQUE. Jean Vague, Chef de clinique a la faculté de médicine de Marseille, and Jean Dunan, Chef de laboratoire. Preface by Professeur J. Roche. Paper. Pp. 256. Price $1.40. Paris: Masson & Cie, 1940.

CHEMISTRY OF DISEASE. Meyer Bodansky, Ph.D., M.D., Director of the John Sealy Memorial Laboratory and Professor of Pathological Chemistry, University of Texas School of Medicine. Oscar Bodansky, Ph.D., M.D., Lecturer in Biochemistry, Graduate Division, Brooklyn College. Cloth. Pp. 684. Price $8. New York: The Macmillan Company, 1940.